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Abstract

A randomised controlled trial of the probiotic *Bifidobacterium breve* BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: the Probiotics in Preterm infantS (PiPS) trial

Kate Costeloe,[1,2]* Ursula Bowler,[3] Peter Brocklehurst,[3,4] Pollyanna Hardy,[3] Paul Heal,[3] Edmund Juszczak,[3] Andy King,[3] Nicola Panton,[1] Fiona Stacey,[1,2] Angela Whiley,[1] Mark Wilks[1,5] and Michael R Millar[1,5]

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Background: Necrotising enterocolitis (NEC) and late-onset sepsis remain important causes of death and morbidity in preterm babies. Probiotic administration might strengthen intestinal barrier function and provide protection; this is supported by published meta-analyses, but there is a lack of large well-designed trials.

Objective: To test the use of the probiotic *Bifidobacterium breve* strain BBG-001 to prevent NEC, late-onset sepsis and death in preterm babies while monitoring probiotic colonisation of participants.

Design: Double-blind, randomised, placebo-controlled trial.

Setting: Recruitment was carried out in 24 hospitals, and the randomisation programme used a minimisation algorithm. Parents, clinicians and outcome assessors were blinded to the allocation.

Participants: Babies born between 23 and 30 weeks' gestation and randomised within 48 hours of birth. Exclusions included life-threatening or any gastrointestinal malformation detected within 48 hours of birth and no realistic chance of survival.

Interventions: Active intervention: 1 ml of *B. breve* BBG-001 in one-eighth-strength infant formula Neocate® (Nutricia Ltd, Trowbridge, UK), (6.7×10^7 to 6.7×10^9 colony-forming units) per dose administered enterally. Placebo: 1 ml of one-eighth-strength infant formula Neocate. Started as soon as practicable and continued daily until 36 weeks' postmenstrual age.

Main outcome measures: Primary outcomes were an episode of bloodstream infection, with any organism other than a skin commensal, in any baby between 72 hours and 46 weeks' postmenstrual age; an episode of NEC Bell stage ≥ 2 in any baby; and death before discharge from hospital. Secondary outcomes included stool colonisation with *B. breve*.

Results: In total, 654 babies were allocated to receive probiotic and 661 to receive placebo over 37 months from July 2010. Five babies were withdrawn; 650 babies from the probiotic group and 660 from the placebo group were included in the primary analysis. Baseline characteristics were well balanced. There was no evidence of benefit for the primary outcomes {sepsis: 11.2% vs. 11.7% [adjusted relative risk (RR) 0.97, 95% confidence interval (CI) 0.73 to 1.29]; NEC Bell stage $\geq$ 2: 9.4% vs. 10.0% [adjusted RR 0.93, 95% CI 0.68 to 1.27]; and death: 8.3% vs. 8.5% [adjusted RR 0.93, 95% CI 0.67 to 1.30]}. *B. breve* colonisation status was available for 1186 (94%) survivors at 2 weeks' postnatal age, of whom 724 (61%) were positive: 85% of the probiotic group and 37% of the placebo group. There were no differences for subgroup analyses by minimisation criteria and by stool colonisation with *B. breve* at 2 weeks. No harms associated with the interventions were reported.

Limitations: Cross-colonisation of the placebo arm could have reduced statistical power and confounded results; analyses suggest that this did not happen.

Conclusions: This is the largest trial to date of a probiotic intervention. It shows no evidence of benefit and does not support routine use of probiotics for preterm infants.

Future work recommendations: The increasing understanding of the pathogenesis of NEC and sepsis will inform the choice of probiotics for testing and better define the target population. Future Phase III trials should incorporate monitoring of the quality and viability of the intervention and colonisation rates of participants; cluster design should be considered.

Trial registration: Current Controlled Trials ISRCTN05511098 and EudraCT 2006-003445-17.

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List of abbreviations

BOOST	Benefits of Oxygen Saturation Targeting clinical trial
CFU	colony-forming unit
CI	confidence interval
DNA	deoxyribonucleic acid
ESBL	extended-spectrum beta-lactamase
GCP	good clinical practice
ICH-GCP	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice
IMP	investigational medicinal product
MALDI-TOF	matrix-assisted laser desorption/ionisation time of flight
MHRA	Medicines and Healthcare products Regulatory Agency
MRSA	meticillin-resistant *Staphylococcus aureus*
NEC	necrotising enterocolitis
NETSCC	NIHR Evaluation, Trials and Studies Coordinating Centre
NPEU	National Perinatal Epidemiological Unit
OR	odds ratio
PCR	polymerase chain reaction
PI	principal investigator
PiPS	Probiotics in Preterm infantS
R&D	research and development
RCT	randomised controlled trial
RR	relative risk
SAE	serious adverse event
SD	standard deviation
SUSAR	suspected unexpected serious adverse reaction
TOS	Trypticase peptone oligosaccharide
VRE	vancomycin-resistant enterococci

Plain English summary

Infection contracted after birth and necrotising enterocolitis (NEC), which is the most common serious complication affecting the gut, are important causes of death and life-long health problems for premature babies. It is thought that giving 'good bacteria' (probiotics) might strengthen the bowel wall and provide protection by preventing bacteria that cause disease from entering the body.

Previous trials of probiotics have provided encouragement, particularly when the results of different trials are added together, but there are concerns about the reliability of some of the trials.

This trial aimed to overcome problems of earlier trials, in particular by being big enough to give clear answers.

The trial was successfully completed and results are available for 1310 babies, born more than 9 weeks early, in 24 different hospitals; 650 were allocated to receive probiotics.

No problem was reported with safety but neither was there any evidence of benefit associated with giving this probiotic to these babies in preventing NEC, severe infection, death or any of the other common problems of prematurity.

We believe that our results support the view that different types of probiotic may have different effects and that it may be a mistake to combine the results of trials of different probiotics as if they were all the same. Although short-term safety is good, we do not yet know about longer-term effects of these products on child development or illnesses such as asthma and, until we know more, we should be cautious about their use.

Scientific summary

Background

Necrotising enterocolitis (NEC) and late-onset sepsis remain important causes of mortality and morbidity in the preterm infant. The postnatal acquisition of diverse bowel flora is delayed in the preterm infant; this may contribute to reduced barrier properties of the intestinal mucosa, making invasion and/or translocation of the bowel wall by potentially pathogenic bacteria more likely. The hypothesis underpinning studies of probiotic administration in preterm babies is that, by encouraging the bowel flora to resemble that of a healthy breast-fed full-term infant more closely, barrier function will be improved and the incidence of late-onset sepsis and NEC will be reduced. The most recent Cochrane review of this topic includes 20 randomised trials with > 5500 participants. The meta-analysis suggests that probiotics do not reduce sepsis but are associated with statistically significant reductions in NEC incidence and all-cause mortality; no adverse events were reported in any trial and the recommendation was made that probiotics should be given routinely to preterm infants, including those whose birthweight is < 1 kg. Despite this, the use of probiotics is variable. Concern has been expressed about the rigour of a number of the published trials, the heterogeneity of the participants, particularly in respect of exclusions and rates of NEC and death in the placebo groups, and the wide range of interventions. These live microbial products are likely to cross-colonise babies, yet none of the trials systematically reports stool colonisation by the administered strains in either the active intervention or placebo groups. This leaves clinicians uncertain about the benefit of routine use and with little guidance as to choice of product.

Objective

To evaluate efficacy and safety of *Bifidobacterium breve* strain BBG-001 to reduce NEC incidences, late-onset sepsis and death in an unselected population of preterm infants in England.

Design

Double-blind, placebo-controlled trial.

Setting

Hospitals with tertiary or secondary neonatal intensive care units in and around London. Recruitment took place in 24 hospitals and continuing care prior to the initial discharge from hospital in a further 33 hospitals.

Participants

Babies born between 23^{+0} and 30^{+6} weeks' gestation were randomised within 48 hours of birth. The randomisation program used a minimisation algorithm to ensure balance on hospital, sex, gestational age and whether or not randomisation occurred within 24 hours of birth. Multiple births were randomised individually. Those with potentially lethal malformations or any gastrointestinal malformation apparent within 48 hours of birth or no realistic chance of survival were excluded.

Interventions

The active intervention, *B. breve* BBG-001, was provided in single-dose sachets as a powder, freeze-dried with maize starch. The placebo was maize starch alone provided as an identical powder in identical sachets. The interventions were suspended in 3 ml of one-eighth strength of the 'elemental' formula Neocate® (Nutricia Ltd, Trowbridge, UK), the maize starch allowed to settle and 1 ml of the supernatant, estimated to contain 6.7×10^7 to 6.7×10^9 colony-forming units of *B. breve* BBG-001, administered daily. The interventions were started as soon as practicable after randomisation, whether or not enteral feeding had begun, and were continued until 36 weeks' postmenstrual age.

Feeding and clinical care including withholding of the intervention was at the discretion of local clinicians.

Stools at 14 days' postnatal and 36 weeks' postmenstrual age were cultured for *B. breve* using a selective medium provided by the manufacturer. If enough stool was available, the 14-day sample was additionally analysed using a strain-specific quantitative real-time polymerase chain reaction.

Main outcome measures

Primary outcomes

1. Any baby with an episode of bloodstream infection, with any organism other than a skin commensal, more than 72 hours after birth and before 46 weeks' postmenstrual age.
2. Any baby with an episode of NEC Bell stage ≥ 2.
3. Death before discharge from hospital.

Secondary outcomes

Secondary outcomes included the composite of the primary outcomes, a range of microbiological outcomes including antimicrobial usage and stool colonisation with *B. breve* and antibiotic-resistant pathogens, time to full enteral feeding, weight gain to 36 weeks' postmenstrual age and major neonatal morbidities.

Statistical power

At a two-sided significance level of 5%, a trial of 1300 infants would have 90% power to detect a 40% relative risk (RR) reduction from 15% to 9.1% for each of the primary outcomes. If the outcomes were less frequent, then the trial would have 90% power to detect a 44% RR reduction from 12% to 6.7% or from 10% to 5.6%.

Results

Recruitment continued for 37 months from July 2010; 654 babies were allocated to receive probiotic and 661 placebo. Consent to use data was withdrawn for five babies, and 650 infants in the probiotic group and 660 in the placebo group were included in the intention-to-treat analysis.

Baseline characteristics were well balanced: the overall median gestation age was 28 weeks (48% <28 weeks); the median birthweight was 1010 g (49% <1000 g); 91% were exposed to antenatal corticosteroid; 36% were exposed to maternal antibiotics within 24 hours of birth; 53% were delivered by caesarean section; 25% were recruited in the first 24 hours after birth; and the intervention was started at a median age of 44 hours. At 14 days, 96% of those infants who were still alive had received some maternal breast milk, augmented in 48.5% with either donor breast or formula milk.

All comparative analyses were adjusted for sex, gestational age and randomisation within 24 hours of birth. Allowance was made for correlations between multiple births. The primary analysis by intention to treat showed no evidence of benefit for any of the primary outcomes {sepsis: 11.2% vs. 11.7% [adjusted RR 0.97, 95% confidence interval (CI) 0.73 to 1.29]; NEC Bell stage ≥ 2: 9.4% vs. 10.0% [adjusted RR 0.93, 95% CI 0.68 to 1.27]; and death: 8.3% vs. 8.5% [adjusted RR 0.93, 95% CI 0.67 to 1.30]}.

Of those surviving at 2 weeks' postnatal age, *B. breve* colonisation status was available for 1186 (94%). In total, 724 (61%) infants were positive: 85% of the active intervention group and 37% of the placebo group.

Subgroup analyses by colonisation status, sex, gestational age as per minimisation, birthweight ($\geq$1000 or $<$ 1000 g), birth ($<$ 28 weeks' gestational age or $\geq$ 28 weeks' gestational age) and randomisation within 24 hours of birth suggested reduced sepsis rates in those born at 28 or 29 weeks [odds ratio (OR) 0.39, 95% CI 0.16 to 0.96], but no other differences for any of the primary outcomes.

Secondary outcomes

There were no differences between the groups for secondary outcomes (apart from *B. breve* colonisation) including the composite outcome of late-onset sepsis, NEC or death. One or both of the stool sampless collected from 38 out of 611 (6.2%) infants in the probiotic group and 35 out of 619 (5.7%) in the placebo group were colonised with antibiotic-resistant bacteria.

In the probiotic group, colonisation was more likely with each week of increasing gestation (OR 1.36; $p < 0.0001$) and less likely in those given any antibiotic between days 6 and 14 (OR 0.26; $p = 0.0027$).

In a secondary non-random analysis, among those with *B. breve* colonisation status known at 2 weeks, there were trends towards fewer babies with primary outcomes associated with colonisation, but none of the findings was statistically significant.

The interventions were well tolerated; there were no positive cultures of *B. breve* from any normally sterile site and no adverse events related to the interventions were reported.

Throughout the recruitment period, the number of viable organisms in the intervention declined slowly, but remained in the expected range and no contaminants were detected.

Conclusions

We believe that the population recruited into this trial is representative of the total population in this geographic area at risk of NEC and late-onset sepsis. It is the first completed trial performed in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice standard and to have systematically studied stool colonisation in all trial centres. The trial has adequate statistical power and the use of a product containing a single bacterial strain provides a clear result.

Although confirming the short-term safety of probiotic interventions, this trial provides no evidence that this particular product is associated with advantage in this population of babies. This result supports the view that it is necessary to assess the efficacy of different probiotic strains in different clinical situations and challenges the validity of combining trials using different probiotic interventions in meta-analyses.

Implications for clinical practice

The results of this trial provide no evidence that supplementation with *B. breve* BBG-001 would affect the risk of late-onset sepsis, NEC or death in this population.

Implications for research

We find no evidence that further trials should be undertaken of this probiotic in this population.

The results of this trial have implications for the design of trials of other probiotic interventions:

- Colonisation rates of both the active and placebo groups should be monitored throughout the trial.
- Cluster design should be considered to reduce any confounding effects of cross-colonisation.

Future work recommendations

The increasing understanding of the pathogenesis of NEC and late-onset sepsis will inform the choice of probiotics for testing and better define the target population. Future Phase III trials should incorporate monitoring of the quality and viability of the intervention and colonisation rates of participants; cluster design should be considered.

Trial registration

This trial is registered as ISRCTN05511098 and EudraCT 2006-003445-17.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research.

Chapter 1 Introduction

This is a multicentre, double-blind, randomised, placebo-controlled trial to study possible benefits of the early administration of the probiotic *Bifidobacterium breve* strain BBG-001 to babies born before 31 weeks' gestation and recruited within 48 hours of birth. The primary end points are late-onset bloodstream infection diagnosed on a sample drawn after 72 hours, necrotising enterocolitis (NEC) and death. The trial aimed to recruit 1300 babies from approximately 20 UK neonatal units.

Chapter 2 Scientific background

Acquired infection and necrotising enterocolitis in preterm babies

Hospital-acquired infection is reported in about 25% of babies with birthweight < 1500 g who survive the first 3 days,[1] and it contributes to the high mortality and morbidity in this population.

Necrotising enterocolitis is the most common serious gastrointestinal complication of preterm birth and has high mortality and morbidity.[2,3] The pathogenesis of NEC is multifactorial,[4] related to immaturity of the immunological and barrier functions and involving bacterial invasion of the intestinal mucosa. There is no agreed international diagnostic case definition for NEC; the system most widely used is the 'modified' Bell classification,[5] which involves a range of clinical, haematological and radiological criteria. Bell stage 1 is very non-specific, whereas Bell stages 2 and 3 have more objective radiological features and are often considered interchangeable with the terms 'proven, confirmed or serious' NEC used in some publications. Estimates of the incidence of 'confirmed' NEC in babies with a birthweight of < 1500 g vary between about 6% and 10%.

The microbiome in the newborn baby and its relation to late-onset sepsis and necrotising enterocolitis

The microbiome is the term used to describe the population of micro-organisms with which individual humans co-exist, predominantly within the bowel. Healthy breastfed term infants become colonised early in life with a wide range of bacteria dominated by bifidobacteria and lactobacilli acquired during and after birth from close contact with the mother;[6] these microbes are believed to confer a range of health benefits. By comparison, preterm infants nursed in neonatal units become colonised with a more limited range of bacteria and fungi.[7–9] The pattern of colonisation reflects the micro-organisms found in the 'antibiotic-rich' environment of the neonatal unit and is dominated by members of the Enterobacteriaceae family, *Pseudomonas*, enterococci, yeasts, staphylococci and clostridia that are potentially pathogenic and may cause infection in the colonised infant or may spread and cause disease in other infants.

The specific mechanisms by which anaerobic lactobacilli and bifidobacteria protect against infection with pathogenic organisms are believed to involve increased secretion of immunoglobulin A and upregulation of immunoglobulin A receptor sites, strengthening of epithelial tight junctions, lowering the intraluminal pH through acid fermentation and modification of intestinal inflammatory responses through preferential stimulation of T-helper cells, all resulting in reduced bacterial translocation.[10] This subject has been the focus of a number of reviews.[11–13]

The extent to which abnormal patterns of early colonisation of the intestine have deleterious effects on later health is also incompletely understood. A number of recent studies have shown changes in the patterns of stool bacterial colonisation in the period preceding the clinical onset of NEC and late-onset sepsis,[14–17] but whether or not these changes are causative or part of the disease process is unclear.

For the context of this trial a probiotic is defined as a live microbial supplement that colonises the gut and improves health.[18]

The extent to which intestinal colonisation with probiotic bacteria can be achieved in the preterm newborn baby is unclear. However, the concept of active management of the bowel flora to prevent hospital-acquired infection and NEC is an attractive therapeutic option that seems likely to have a good safety profile.

Probiotics and the prevention of late-onset sepsis and necrotising enterocolitis

Randomised controlled trials

When the Probiotics in Preterm infantS (PiPS) trial was designed, we believed that only one randomised controlled trial (RCT) had been published that reported the effect of probiotics on late-onset sepsis and/or NEC in preterm babies. This was an Italian trial published in 2002 and involving 585 babies below 33 weeks' gestational age[19] treated with a product containing *Lactobacillus rhamnosus* GG. It failed to show a significant reduction in NEC incidence or blood culture-positive episodes of late-onset sepsis (probiotic vs. placebo: NEC, 1.4% vs. 2.7%; septic episodes, 4.1% vs. 4.7%). The results are difficult to interpret, as the analysis was not by intention to treat; babies dying in the first 2 weeks were excluded and only septic episodes and episodes of NEC with onset at least 7 days after commencement of the intervention were considered in the analysis. This probably accounts for the low reported rates of adverse outcomes.

When recruitment to the PiPS trial began in 2010, four[20–23] further trials with clinical primary outcomes had been published.

The first two trials were reported in 2005 and showed a reduction in the incidence of NEC in infants given probiotic mixtures; both studies recruited at a single site. The first was from a hospital in Taiwan,[20] in which a mixture of *L. acidophilus* and *B. infantis* or placebo was given in breast milk twice daily until discharge from hospital to 367 babies of birthweight < 1500 g, who had survived beyond 7 days and were clinically stable with umbilical lines removed and commencing milk feeds. Reductions in incidence were seen for NEC, from 5.3% to 1.1% ($p = 0.04$); blood culture-positive late-onset sepsis, from 19.3% to 12.2% ($p = 0.03$); death, from 10.7% to 3.9% ($p = 0.009$); and for the combined outcome of NEC, late-onset sepsis or death from 32.1% to 17.2% ($p = 0.009$). The second study[21] recruited 145 babies at an Israeli hospital, who were randomised to receive a product containing three probiotic strains (*B. infantis, Streptococcus thermophilus* and *B. bifidus*) or unsupplemented milk feeds given once a day. The median age at commencement of the intervention was 3 days and the intervention was continued until 36 weeks' postmenstrual age. There was no difference in episodes of blood culture-positive infection or of death, but episodes of NEC appeared to be reduced in the intervention arm (16.4% vs. 4.0%; $p = 0.03$) and it was reported that there was a reduction in the severity of the illness. These two studies, the first to report prevention of NEC, were subject to considerable interest and extensive review.[24–26]

In 2007 a meta-analysis[27] was published including these three trials[19–21] together with four others designed to study different outcomes, one of which involved a fungal rather than a bacterial intervention.[28] A total of 1393 babies were involved, and the conclusion was that there was evidence that probiotic interventions might reduce the incidence of NEC and all-cause mortality, apparently without adverse effects, but that there were important outstanding questions about choice of probiotic product and dosing.

The third and fourth RCTs with clinical primary outcomes were published in 2008 and 2009, a single-site trial from a hospital in India[22] and a multicentre trial from Taiwan.[23] In the trial from India, a combination of *B. infantis, B. bifidum, B. longum* and *L. acidophilus* given with breast milk twice daily to 186 babies of < 32 weeks' gestation and birthweight < 1500 g was compared with breast milk alone. Participants were clinically stable and, as in the previous studies, receiving milk feeds. The end points included feed tolerance, length of stay and serious neonatal morbidities. Babies dying from causes other than late-onset sepsis or NEC were excluded and no power calculation was given. A significant reduction in time to achieve full feeds and length of stay was reported to be associated with probiotic use. There was an overall reduction in all stages of NEC from 15.8% in the control group to 5.5% in the probiotic group ($p = 0.04$), but no significant reduction in the incidence of NEC of Bell stage ≥ 2. There was a significant reduction in culture-positive late-onset sepsis, from 29.5% to 14.3% ($p = 0.02$), and of death, from 14.7% to 4.4% ($p = 0.04$).

The multicentre trial from Taiwan[23] recruited a total of 434 babies of birthweight < 1500 g and gestational age < 4 weeks from seven centres. A product containing *B. bifidum* and *L. acidophilus* was added to the milk feed; babies entirely fed with formula were excluded, as were babies in whom feeds had not been started by 3 weeks of age. There was a composite primary outcome, death or NEC Bell stage ≥ 2, which was significantly lower in the intervention group (1.8% vs. 9.2%; $p = 0.002$). In addition, there were more babies with late-onset sepsis in the intervention group (18.4% vs. 11.1%.) A large number of infants died (98 out of a potential 580 eligible infants) without ever achieving the study entry criteria.

In the period leading up to the start of recruitment to the PiPS trial in 2010 and during recruitment there have been sharp divisions in the paediatric literature about the use of probiotics. Some authors have strongly advocated a change of practice to routine use[29] because of the apparent association with a reduction in NEC and death, as suggested in a series of meta-analyses,[30–32] whereas others have recommended caution because of the heterogeneity of the participants and of the interventions and the methodological failings of some trials.[33,34]

At the time of writing, 11 RCTs designed to study the efficacy of a bacterial probiotic intervention, with late-onset sepsis and/or NEC and/or death as the primary outcome, have been published in English. These trials account for 4396 of the 5529 (80%) babies randomised in 20 trials included in the most recent Cochrane review of probiotics to prevent NEC in preterm babies.[35] These trials are characterised by a range of inclusion and exclusion criteria and by varying exposure to maternal breast milk. This may, in part, explain the wide reported ranges of rates of late-onset sepsis, NEC and death. Of those studies reporting such data, mortality and NEC rates among excluded infants are in some cases high (*Table 1*). The extent to which reviews such as this may be subject to publication bias is difficult to assess owing to the inclusion of a large number of trials. Many of which are small and not designed to study clinical outcomes.

With the exception of a multicentre trial published in 2012 and recruiting babies of birthweight up to 2000 g,[39] the probiotic intervention was given either in milk or, in one study,[42] separately but coincident with the start of feeding. This suggests that babies with perceived contraindications to starting feeds, who are likely to be those babies at highest risk of NEC, might be excluded or have deferred entry to the trials. The majority of the trials were not placebo controlled and relied on the responsible clinical staff being blind to the allocation through the use of unsupplemented milk as the comparator (see *Table 1*).

None of the trials was designed with statistical power to study NEC or death rates as separate outcomes.

In contrast to NEC and death, the various meta-analyses do not suggest a protective effect of probiotics for late-onset sepsis. The assessment of efficacy to reduce late-onset sepsis is complicated by the lack of a standardised definition of the outcome.

This problem was addressed by the multicentre Australasian ProPrems trial,[41] which is the largest of the previously published trials. The results were presented in 2012 but were not published until after the completion of PiPS trial recruitment. A rigorous definition of late-onset sepsis was used and the trial was statistically powered to show a reduction from 23% to 16%. The event rates of both late-onset sepsis and NEC in this trial were lower than predicted and a non-statistically significant reduction in late-onset sepsis, from 16.2% to 13.1%, was observed.

It is generally held that, in order for a probiotic intervention to be effective, it should 'colonise' the intestine and the administered bacteria should multiply within it. Successful colonisation is likely to be influenced by local factors such as feeding and antimicrobial use. Human breast milk contains oligosaccharides known to promote colonisation by bifidobacteria while many probiotic strains are sensitive to commonly administered antimicrobials, for example bifidobacteria are sensitive to penicillins. Manufacturers of probiotics inevitably select strains that readily colonise the intestine and, theoretically, these strains might be particularly likely to

TABLE 1 Characteristics of published trials with primary outcomes culture-proven late-onset sepsis and/or Bell stage ≥ 2 NEC and/or death involving a live bacterial intervention

Reference	Eligibility criteria	Exclusions (other than congenital malformations and lack of consent) and their outcomes if available	Intervention including whether or not in milk	Placebo	Blind	Age at starting intervention	Rates of late-onset sepsis, NEC and death in non-intervention group
Dani *et al.* 2002[19]	< 33 weeks old and < 1500 g in weight; randomised, *n* = 585	Death within 2 weeks of birth, *n* = 29	*Lactobacillus* GG with milk	Yes	Yes	Mean 3.4 days (SD 3.7 days)	After 7 days of intervention: late-onset sepsis, 12 of 290; NEC, 8 of 290; death, N/A
Bin-Nun *et al.* 2005[21]	< 1500 g in weight; randomised, *n* = 148	N/A	*B. bifidus*, *B. infantis* and *S. thermophilus* with milk	No	Yes	Mean 2.7 days[a] (SD 2.3 days)	Late-onset sepsis, 24 of 73; NEC, 12 of 73; death, 8 of 73
[b]Lin *et al.* 2005[20]	> 7 days old, < 1500 g in weight, central lines removed > 24 hours; randomised, *n* = 367	50 of 417 potential recruits died or had NEC < 7 days	*L. acidophilus* and *B. infantis* with breast milk	No	Unclear	N/A, but after 7 days	Late-onset sepsis, 36 of 187; NEC, 10 of 187; death, 20 of 187
Lin *et al.* 2008[23]	< 34 weeks old, < 1500 g in weight 'who survived to feed enterally'; randomised, *n* = 443	Exclusive formula feeds, nil by mouth > 3 weeks, 98 of 580 assessed for eligibility died before milk started	*L. acidophilus* and *B. bifidum* with breast milk	No	Unclear	Mean 4.5 days[a] (SD 3.0 days)	Late-onset sepsis, 24 of 217; NEC, 14 of 217; death, 9 of 217
[c]Samanta *et al.* 2009[22]	< 32 weeks old, < 1500 g in weight, survived 48 hours; randomised, *n* = 186	Died from 'other' illnesses	*B. infantis*, *B. bifidum*, *B. longum* and *L. acidophilus* with breast milk	No	Not known	Mean 6.0 days[d] (SD 1.4 days)	Late-onset sepsis, 28 of 95; NEC, 15 of 95; death, 14 of 95
Mihatsch *et al.* 2010[36]	< 30 weeks old; randomised, *n* = 183	None	*B. lactis* BB12 with milk	Yes	Yes	Mean 5 days (SD 2.7 days)	Late-onset sepsis, 40 of 89; NEC, 4 of 89; death, 1 of 89
Sari *et al.* 2011[37]	< 33 weeks old; < 1500 g in weight;[e] randomised, *n* = 242	None	*L. sporogenes* with milk	No	Unclear	Mean 2 days[d]	Late-onset sepsis, 26 of 111; NEC, 10 of 111; death, 4 of 111
[f]Braga *et al.* 2011[38]	750–1499 g in weight;[g] randomised, *n* = 243	Congenital infection	*B. breve* and *L. casei* with donor breast milk	No	Yes	Day 2	Late-onset sepsis, 42 of 112; NEC, 4 of 112; death, 27 of 112
Rojas *et al.* 2012[39]	≤ 48 hours old, < 2000 g in weight, haemodynamically stable;[h] randomised, *n* = 750	None	*L. reuteri* DSM 17938[i]	Yes	Yes	Day 2	Late-onset sepsis, 40 of 378; NEC, 15 of 378; death, 28 of 378

Reference	Eligibility criteria	Exclusions (other than congenital malformations and lack of consent) and their outcomes if available	Intervention including whether or not in milk	Placebo	Blind	Age at starting intervention	Rates of late-onset sepsis, NEC and death in non-intervention group
Fernández-Carrocera *et al.* 2013[40]	< 1500 g in weight, randomised, *n* = 150	Apgar score of < 6 at 5 minutes, NEC Bell stage 1	*L. acidophilus*, *L. rhamnosus*, *L. casei*, *L. plantarum*, *B. infantis* and *S. thermophilus* with milk	No	Unclear	Median 5 days (range 1–23 days)	Late-onset sepsis, 44 of 75; NEC, 12 of 75; death, 7 of 75
[j]Jacobs *et al.* 2013[41]	< 32 weeks old, < 1500 g in weight, randomised < 72 hours;[k] randomised, *n* = 1099	Likely to die within 72 hours, mother taking non-dietary probiotics	*B. infantis*, *B. lactis* and *S. thermophilus* with milk	Yes	Yes	Median 5 days (IQR 4–7 days)	Late-onset sepsis, 89 of 551; NEC, 24 of 551; death, 28 of 551
[j]Oncel *et al.* 2014[42]	< 33 weeks old, ≤ 1500 g in weight; randomised, *n* = 424[l]		*L. reuteri* DSM 17938[i]	Yes	Yes	Median 1 day (range 1–5 days)	Late-onset sepsis, 25 of 200; NEC, 10 of 200; death, 20 of 200

IQR, interquartile range; N/A, not applicable, SD, standard deviation.

a This is the time of starting feeds; the intervention seems to have been started at the same time.

b It is not explicit, but seemingly the participants in this trial were fed exclusively with either maternal or donor breast milk.

c It is not explicit, but seemingly the participants in this trial were fed exclusively with maternal breast milk.

d These are ages at enrolment.

e One infant with spontaneous intestinal perforation and 16 who died within 7 days of enrolment after randomisation are excluded from the analysis.

f For the first 2 weeks participants were fed exclusively with either maternal or donor breast milk.

g Twelve babies died post randomisation before starting the intervention and are excluded from the analysis.

h Of 2055 babies screened, 1446 met the inclusion criteria and, of these, parental consent was obtained for 750 (58%).

i In both the Rojas *et al.*[39] and Oncel *et al.*[42] trials the intervention was given apart from the milk. In Rojas *et al.*[39] the intervention was started whether or not feeds were started and 80% of babies were exclusively formula fed, and in Oncel *et al.*[42] the intervention was given at the same time as the first feed to babies who survived to be fed.

j These trials were published after the completion of recruitment to the PiPS trial.

k In total, 2520 babies were assessed for eligibility, of whom 1421 were excluded: 234 because they failed to meet the entry criteria and 741 because consent was declined. In 446 cases the reason why babies were not recruited as not reported.

l First-week deaths and babies developing outcomes within 7 days of starting the intervention are excluded from the analysis.

spread between babies, especially in hospitals where cots are close together or the nurse-to-baby ratio is low. In this respect, the efficacy of a probiotic intervention might be expected to vary more between different institutions than a standard chemical drug intervention. Of the 20 RCTs included in the most recent Cochrane review,[35] the only trial to report colonisation by allocated group in detail is the single-site study reported by Kitajima *et al.*,[43] which involved the administration of a single-strain probiotic product containing *B. breve* YIT4010 (BBG-001). Of 45 babies in the probiotic group, 73% were colonised at 2 weeks and 91% at 6 weeks, whereas, of the 46 babies given placebo, 12% were colonised at 2 weeks and 44% at 6 weeks. This adds a level of complexity to the interpretation of all data from trials of probiotics, particularly for those using polymicrobial products, as it is likely that different components will colonise babies in both groups so that at different time points in their clinical course babies might be colonised with anywhere between none or all of the administered strains.

Observational studies/historic comparisons

Despite the frequent calls for probiotics to be used routinely for preterm babies, there are few published accounts of their impact in routine use.

An early report[44] of a trial in a tertiary hospital in Colombia, in which a product containing *L. acidophilus* and *B. infantis* was given for 1 year to all admitted newborn infants, reported a decrease in all stages of NEC compared with the previous year, from 6.6% ($n = 1237$) to 3.0% ($n = 1282$); all other aspects of care were unchanged.

There have been more recent reports of use targeted towards preterm babies.

In 2010 Luoto *et al.*,[45] reported on 12 years' experience in five tertiary neonatal units in Finland. In one neonatal unit, following an outbreak of NEC, administration of *Lactobacillus* GG was introduced for all babies of birthweight < 1500 g. In three other neonatal units, the same product was administered to babies with gastrointestinal problems and the final neonatal unit used no probiotic. The standard of care in all hospitals was to use donor breast milk in the absence of maternal milk. The authors did not find a protective effect in the hospital using 'prophylactic' probiotics when the incidence remained higher than in the other hospitals or any effect on the clinical course of NEC in those hospitals in which probiotics were given to symptomatic babies.

A further three retrospective cohort studies have been published.[46–48]

A report from a single site in the USA[46] compared the incidence of NEC in 79 babies (of birthweight ≤ 1000 g) born between 2009 and 2011 in whom *L. reuteri* was routinely administered and babies born in the previous 5 years; detailed feeding data were not reported and infants who died in the first week were excluded. A reduction in NEC Bell stage ≥ 2 from 15.1% to 2.5% was reported ($p = 0.0475$); there were baseline differences in the characteristics of the babies and between-year variation in NEC incidence.

In a study in France,[47] during a 3-year period from 2008, babies born between 24 and 31 weeks' gestation ($n = 347$) and starting feeds within 48 hours of birth on a tertiary neonatal unit were administered *L. casei rhamnosus*, Lcr35 strain from the beginning of feeding, their outcomes were compared with those of unsupplemented babies born in the previous 5 years ($n = 783$). Babies dying in the first week were excluded from the analysis.[47] In the second period, the incidence of late-onset sepsis was reduced from 16.6% to 10.7% [odds ratio (OR) 0.60, 95% confidence interval (CI) 0.40 to 0.89], the incidence of NEC Bell stage ≥ 2 was reduced from 5.3% to 1.2% (OR 0.23, 95% CI 0.08 to 0.69) and the mortality rate fell from 4.8% to 2.3% (OR 0.46, 95% CI 0.21 to 1.00).

A Canadian study[48] published in 2014 reported NEC Bell stage ≥ 2 and the composite outcome NEC or death of babies born before 32 weeks' gestation for 17-month periods before ($n = 317$) and after ($n = 294$) the introduction of a product containing *B. breve, B. longum, B. bifidum, B. infantis* and *Lactobacillus* GG was given with the first feed at a single site. The incidence of NEC decreased from 9.8% to 5.5% ($p < 0.02$) and the incidence of death or NEC fell from 17.0% to 10.5% ($p < 0.05$). There was a non-significant reduction in death as a separate outcome. After adjustment for gestational age, intrauterine growth restriction and sex, the OR for NEC in the second period was 0.51 (95% CI 0.26 to 0.98) and for death or NEC was 0.56 (95% CI 0.33 to 0.93).

Safety

None of the published RCTs or non-randomised studies, including a summary of 6 years' use of *Lactobacillus* GG across two neonatal units in northern Italy[49] that contains no efficacy data, reports any complications of probiotic administration; most importantly, no instances were recorded of late-onset sepsis with the administered probiotic strains.

Before the beginning of recruitment to this trial there had been occasional reports, including in the paediatric literature,[50,51] of disseminated infection following enteral supplementation with *Lactobacillus* species, but no reports of late-onset sepsis with *Bifidobacterium*. In 2010, what we believe to be the first case of a *Bifidobacterium* septicaemia was reported.[52] This involved a positive blood culture for *B. breve* strain BBG-001 (the strain used for the PiPS trial) in a full-term baby recovering after surgery for exomphalos. The baby is described as having a mild illness that was treated with standard empirical antibiotic treatment involving ampicillin/sulbactam and amikacin; the child made an uneventful recovery.

In 2012, a second report[53] described a twin born at 27 weeks' gestation, birthweight 600 g, who was fed with maternal breast and in whom a probiotic preparation containing *B. infantis* and *L. acidophilus* was instituted on day 8. On day 18 the infant became unwell with abdominal symptoms. A blood culture grew two species, *B. infantis* and *B. longum.* She was treated with vancomycin, cefotaxime and metronidazole, and recovered.

There is an anxiety that, theoretically, manipulating the developing microbiome by administering probiotics might modify the immunological function of the intestine or that antibiotic resistance genes might be transferred from the probiotic to pathogenic bacteria, thereby putting the individual at increased short-term risk of infection or possibly of unpredictable long-term health change.[54]

Studies on the effect of probiotics on intestinal colonisation with potential pathogens are few, and the results inconsistent. A small study involving 30 babies, with a mean gestational age of 33 weeks and a mean birthweight of 1486 g, was suggestive that *B. breve* administration might be associated with reduced colonisation with Enterobacteriaceae.[55] However, a more recent placebo-controlled randomised trial[56] of formula-fed babies, born before 32 weeks' gestation, found increased colonisation with Enterobacteriaceae, enterococci and staphylococci in 21 of 47 babies whose feed was supplemented with *Lactobacillus* GG. This was not associated with increased late-onset sepsis in these babies. Of the 12 clinical trials listed in *Table 1*, all of which were designed to study clinical outcomes, five reported higher rates of sepsis in the active arm than in the placebo arm,[19,21,23,37,39] although only one was statistically significant.[23] These trials use various definitions of late-onset sepsis.

A RCT studying a product with six bacterial strains, four species of *Lactobacillus* and two of *Bifidobacterium*, in 296 adult patients with acute pancreatitis reported increased mortality in the active arm, [24/152 (16%) vs. 9/144 (6%) in the placebo arm ($p = 0.01$)].[57] The most frequent cause of death was multiorgan failure and there were no reports of probiotic bacteraemias. In 9 of the 24 patients in the active group who died, ischaemic bowel was found at either laparotomy or autopsy; ischaemic bowel was not found in those patients in the placebo group who died. The intervention in this trial was given twice daily directly into the

jejunum and represents a huge bacterial load, estimated at 10^{10} bacteria. The reasons for the increased mortality are unclear. This trial might be interpreted as a reminder that, despite worldwide extensive consumption of probiotics, it should not be assumed that they are safe in extremely ill patients with compromised intestinal function; this would include preterm babies, particularly those with problems establishing enteral nutrition.

The justification for continued recruitment to the PiPS trial was kept under review throughout its progress as reports of more trials of routine use and of probiotic septicaemias became available; at no time was it considered that the accumulating evidence either of efficacy or safety was such that a recommendation to stop the trial early should be made. The overarching consideration was whether or not the findings of the various trials were applicable to the population of preterm babies at risk of late-onset sepsis and NEC in UK neonatal units. Rates of late-onset sepsis and NEC are inversely related to gestational age at birth[2,58–60] and become relatively low from around 32 weeks' gestation; the requirement of clinicians is for a preventative intervention that can safely be given to all babies at risk of late-onset sepsis and NEC.

The choice of probiotic

The ideal probiotic for a clinical trial would have extensive preclinical data, including experience in the preterm newborn infant and information about dosage, supporting its probable efficacy and safety. In addition, it would be available in a stable pure form known to be free of contaminants; a suitable and indistinguishable placebo would be available; and it would be easy to grow and identify the bacterium in the laboratory so that colonisation of participants could be monitored and probiotic infection easily detected. None of the interventions used in the published studies in the newborn infant fulfils these criteria.

There are also choices to be made regarding whether or not a single or multistrain product is used.

There are very few studies comparing different probiotic interventions in the preterm baby. One recent Phase 1 study[61] suggested different effects of a *B. infantis* species compared with *B. longum* in respect of bacterial diversity and total counts of bifidobacteria, particularly when augmented by administration of maternal milk. A second study[62] comparing a product containing a single strain of *B. breve* with a product providing the same quantity of *B. breve* together with two species of *B. longum* suggested that the three-strain product was associated with increased colonisation with *B. breve* and fewer Enterobacteriaceae species; whether or not these effects are attributable to the diversity or to the greater bacterial load of the three-strain product is unclear.

Of the 12 trials included in *Table 1*, four used products containing a single strain, three contained different strains of *Lactobacillus*, one contained *B. lactis* BB12 and the remaining four studies used combinations of up to six different bacterial strains. In large part, the choice of product appears to have been governed by availability and the ability to mix it with milk.

Although not explicitly stated in the text, in one trial conducted in Israel by Bin-Nun *et al.*,[21] and reported at a scientific meeting (Dr C Hammerman, Zedek Medical Center, Jerusalem, personal communication), the intervention [ABC Dophilus® (Solgar®), which contained *B. infantis*, *S. thermophilus* and *B. bifidus*], was selected because of anxiety about possible infection with *Lactobacillus*-containing products. The same product was used in the recent and heretofore the largest published trial, ProPrems, carried out in Australia.[41] When asked about the choice, the author explained that it was not because of the bacterial content but simply that it had been previously evaluated and shown to have efficacy against NEC incidence, was available and could be imported under licence into Australia (Dr SE Jacobs, Royal Women's Hospital, Melbourne, VIC, personal communication). The formulation of that product has now changed so that it contains *Lactobacillus*.

Two published meta-analyses[34,63] attempt to group trials to study the effects of different organisms and combinations, but they fail to reach clear conclusions and highlight the need for further study.

Quality

Of the 12 trials detailed in *Table 1*, nine quote the manufacturer's data describing the content of the product but do not describe the storage conditions or any further testing to confirm the contents, their purity or their stability through the course of trial recruitment. The *B. lactis* used by Mihatsch *et al.*[36] was checked monthly to ensure the viability and purity of the product together with the 24-hour stability of the prepared suspension. The six-strain product used by Fernández-Carrocera *et al.*[40] (four strains of *Lactobacillus*, one of *B. infantis* and one of *S. thermophilus*) was checked twice against the manufacturer's quality control register and the ABC Dophilus used for the ProPrems trial[41] was imported under licence into Australia. Each batch was then subjected to independent confirmation of taxonomy and quality by checking the probiotic content using polymerase chain reaction (PCR) and the purity by culture.

Experience with *Bifidobacterium breve* BBG-001

Use of *B. breve* BBG-001, the probiotic strain used for the PiPS trial, was first reported by a Japanese group.[43] Ninety-one infants of birthweight < 1500 g were randomised to receive active product or placebo. The trial commenced with milk feeds, administered twice daily and continued for 28 days, by which time 82% of the active intervention group and 28% of the placebo group were colonised with *B. breve* BBG-001. Clinical outcomes analysed whether or not the baby was successfully colonised with the probiotic organism. Improved food tolerance, accelerated time to establish full feeds and increased weight gain that was sustained after discontinuation of administration of probiotic were reported. No other clinical outcome was published and none is available; probiotic use with this product became and remains routine in that investigator's department (Dr Kitajima, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, 2013, personal communication).

A single-site pilot study using the same product, *B. breve* BBG-001, was undertaken by the current investigators.[64] *B. breve* BBG-001 was used because at the time the study was designed it was the only probiotic strain reported to confer any clinical benefit in the preterm baby.[43] The primary objectives of the pilot were to study whether or not the intervention was tolerated this early in development and to confirm that colonisation was achieved with a once-daily dosage regimen. The design differed from previous studies in that the study product was commenced within 48 hours of birth, whether or not milk feeds had been started. This was to avoid excluding those babies at greatest risk of adverse outcomes and to maximise the possibility of early colonisation with the probiotic organism, even in babies from whom the responsible clinician might choose to withhold milk feeds because of a perceived high risk of NEC. The products were prepared as described in the report by Kitajima *et al.*,[43] but only a single 1-ml dose was given, as opposed to the whole content of the sachet given in two or three 1-ml doses. We estimated the dose given to be around 5×10^8 colony-forming units (CFUs) of *B. breve* BBG-001. The colonisation rates we achieved were similar to those quoted by Kitajima *et al.*[43] and the numbers of bifidobacteria in the stools of those babies who were colonised were the same whether they were in the active intervention or placebo groups: at 14 days, 12 out of 19 (63%) infants in the active intervention group were colonised with a mean 7.3 [standard deviation (SD) 1.7] $\log_{10}$ CFUs per gram wet weight of stool and 4 out of 17 (24%) infants in the placebo group were colonised with a mean with 7.4 (SD 3.0) CFUs per gram wet weight of stool. These data support the conclusion that *B. breve* had actively colonised the babies and the same dose was therefore used in the main trial.

Forty infants of birthweight < 1500 g were randomised at a single site (Homerton University Hospital Foundation Trust, London, UK) over a 6-month period in 2004 to receive *B. breve* BBG-001 or placebo; both products were well tolerated by all babies. Quantitative microbiology was undertaken on stools. Analysis of the stool passed closest to 28 days showed that 79% of the group receiving probiotic and 35% receiving placebo were colonised with *B. breve* BBG-001; this high cross-contamination rate is comparable with published experience[43] and was considered likely to have occurred both in the milk kitchen and between babies in the ward. All babies who commenced enteral feeding did so with maternal breast milk.

Analysed by intention to treat, probiotic supplementation was associated with improved feed tolerance and weight gain, and there were fewer babies with episodes of infection at 28 days (23% vs. 44%). The study was too small to allow any estimate of an impact on the incidence of NEC (nine suspected or proven cases, of whom five were randomised to receive probiotic and four placebo). When outcomes were analysed by whether or not the infant was colonised with the administered probiotic, it was found that colonisation was associated with a reduction in the number of babies with any episode of infection over the entire hospital stay (from 66% to 24%; $p = 0.017$) and also that fewer colonised babies remained oxygen dependent at 36 weeks' postmenstrual age (40% vs. 79%; $p = 0.038$). In addition, there was some evidence of increased microbial diversity in the stools of colonised babies, although the numbers are small. In particular, at 28 days, no stool of non-colonised babies was also colonised with anaerobic bacterial species; in contrast, 66% of those colonised with *B. breve* BBG-001 were also colonised with anaerobic bacterial species. Colonisation with Gram-negative organisms was high in both groups. When analysed by intention to treat, there was no difference in the duration of antibiotic use in the two groups, but, when analysed by whether or not there was successful colonisation, there was a significant reduction in the number of days on antibiotics over the whole hospital stay in those colonised, from a mean of 39 days to 19 days ($p = 0.04$).

The intervention was continued for a shorter time period (28 days) in this pilot study than in the subsequently published studies that found a reduction in NEC incidence with probiotic use, all of which continued the intervention to either 36 weeks' postmenstrual age or discharge from hospital. Two babies randomised to receive probiotic in the pilot study developed proven NEC that was fatal: one at 29 days and one at 30 days (one of these infants was not successfully colonised). When the PiPS trial was designed it was recognised that babies at high risk of developing NEC may do so later than 4 weeks' postnatal age, it is now known from observational studies that more immature babies develop the disease at an older postnatal age with the peak age at onset around 31–34 weeks' postmenstrual age.[65,66]

Subsequent to the design of the PiPS trial, we are not aware of any published reports of the use of *B. breve* BBG-001 in the newborn baby.

Regulatory status of probiotics

At the time of the pilot study using *B. breve* BBG-001 that we conducted in 2004,[64] probiotics were considered as food supplements and the trial was not conducted to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP) standard. A product given to prevent such serious complications of prematurity as NEC, late-onset sepsis and death clearly fulfils the definition of a medicinal product used within the European Community: 'Any substance or combination of substances presented as having properties for treating or preventing disease in human beings' (Article 1, Directive 2001/83/EC).[67] The regulator, the Medicines and Healthcare products Regulatory Agency (MHRA), required that for this trial the probiotic intervention should be considered as a medicine and the PiPS trial, unlike all other published trials investigating the use of probiotics in newborn babies, was conducted to ICH-GCP standard.

Rationale for the design of the Probiotics in Preterm infantS trial

When the PiPS trial was designed, there had been no published trials, apart from the study of Dani *et al.*,[19] that reported effects of probiotics on NEC, late-onset sepsis and death, and no trials reporting benefits of probiotic use. Despite this, in the context of the current understanding of the epidemiology and pathogenesis of NEC and late-onset sepsis and the importance of identifying preventative strategies, the probability that a probiotic intervention might be both efficacious and safe seemed high.

Choice of product

When our trial was designed, as far as we were aware the only study to report any benefit associated with probiotic use in the newborn infant was the trial by Kitajima *et al.*[43] published in 1997, studying nutritional outcome, although the analysis was based on whether or not the baby had been successfully colonised rather than by intention to treat. We were also aware that the product used in that trial, *B. breve* BBG-001, had been in routine use in Japan for a number of years, seemingly without problems. These observations underpinned our decision to use this product for our pilot study, in which we found it to be well tolerated and confirmed that we could achieve good colonisation rates with a single daily dose.

We were keen to monitor colonisation of all participants, and *B. breve* BBG-001 had the further advantage that the manufacturer was able to provide a selective strain-specific medium so that it could reliably be cultured and identified.

Population

The study population was selected as being that at greatest risk of NEC. In particular, we were keen to start the intervention as soon as practicable, not only because babies begin to acquire intestinal flora from birth, whether or not fed, but also because as the clinical course progresses and complications arise we believe that it is easier to find reasons not to recruit babies into trials and we thought it essential that we recruit a trial population that was as representative as possible of all preterm admissions so that at trial conclusion we could address the question of whether or not probiotics should be given routinely to this population.

Chapter 3 Methods

This was a multicentre, double-blind RCT.

Objective

The objective of the trial was to determine whether or not early administration of the probiotic *B. breve* strain BBG-001 to preterm infants reduced the incidence of episodes of infection, NEC and death.

The trial protocol is available on the NIHR Evaluation, Trials and Studies Coordinating Centre (NETSCC) website at www.nets.nihr.ac.uk.

Participants

Participants were preterm babies born between 23^{+0} and 30^{+6} weeks' gestation and recruited with informed signed parental consent within 48 hours of birth. Babies were eligible for recruitment whether or not they had been born in the recruiting centre. Those with a lethal congenital malformation or any malformation of the gastrointestinal tract detected before 48 hours or who were considered to have no realistic chance of survival were excluded. Receiving antibiotics for proven or suspected infection was not an exclusion criterion.

Trial sites

The trial was conducted at 57 sites. Of these, 24 were recruitment sites and 33 were sites to which participants were transferred for continuing care. A complete list of the recruitment sites is available in *Appendix 1*.

Interventions

The active intervention was *B. breve* BBG-001, suspended in one-eighth strength of the infant formula Neocate® (Nutricia Ltd, Trowbridge, UK). The placebo was one-eighth-strength Neocate. A description of Neocate is available at www.neocate.co.uk/uploadedFiles/Neocate/Resources_Library/Documents/Neocate_LCP_data_card.pdf (accessed 26 August 2016).

The probiotic and placebo powders were manufactured and supplied by the Yakult Honsha Co. Ltd (Tokyo, Japan) in identical square foil sachets each containing 1 g of product. The sachets of active product contained *B. breve* BBG-001 freeze-dried with maize starch and those of placebo contained freeze-dried maize starch alone; the appearance of the powders was identical. The trial was conducted using a single batch of products manufactured specifically for this study, the release criteria for this batch stated that each sachet of the active product contained between 2×10^8 and 2×10^{10} CFUs. After importation to the UK, the sachets were packed at Bilcare Global Clinical Supplies (Europe) Ltd into packages each containing 91 sachets (the maximum number of sachets a baby might require) of either active product or placebo. Each of the 91 sachets and the package was labelled with a unique five-digit alphanumeric identifier.

Product preparation, administration and blinding

The manufacturer's instructions involved suspending the powders in water, allowing the maize starch to settle for 30 minutes and administering the supernatant within the next 2.5 hours. Prepared in this way, the supernatant of the active product was cloudy and that of the placebo clear. This was overcome by

substituting one-eighth-strength Neocate for the water. Occasionally the turbidity of the supernatant still varied slightly and, therefore, to be completely confident that the active intervention and placebo were indistinguishable, they were prepared in specially manufactured amber-coloured bijou bottles (*Figure 1*).

Kitajima *et al.*,[43] using the same product, prepared it using 2 ml of water. During our preliminary work we found that when using 2 ml that it was sometimes difficult, using a syringe, to withdraw 1 ml without disturbing the maize starch residue. We were keen not to increase the volume that we were administering to the babies but equally keen to ensure, with minimal evidence to guide us, that we gave adequate numbers of bacteria. By a process of trial and error we found that, if we increased the volume used to suspend the powder to 3 ml, then only rarely were we unable easily to withdraw 1 ml. We emphasised to investigators the importance of not disturbing the maize starch and suggested that, if they had any difficulty withdrawing 1 ml, they could simply give less on that day.

The products were prepared in the milk kitchens on the neonatal units of the participating hospitals, usually by one of the nurses engaged in clinical care. In order to minimise the possibility of cross-contamination of the placebo by *B. breve* BBG-001, members of the trial team provided on-site training with an emphasis on handwashing and decontamination of working surfaces in between preparing each baby's intervention. This teaching was repeated, on request, for new staff and was supported with detailed guidance on laminated sheets for display in milk kitchens. The guidance sheet for product preparation is available in *Appendix 2*.

Dosage

The range of values quoted by the manufacturers of products used in published trials is from 10^6 to 10^9 CFUs per dose. The dose used in the study of Kitajima *et al.*[43] is the most relevant for this trial because the same product was used. The babies in the study of Kitajima *et al.*[43] were given the contents of a whole sachet (estimated in the publication to contain 1×10^9 CFUs) in two or three divided doses (i.e. 3.3×10^8 to 5.0×10^8 CFUs per dose) each 1 ml in volume. In our pilot study we achieved colonisation rates similar to Kitajima *et al.*[43] with a single dose. The manufacturer stated that each sachet of the batch used for the PiPS trial contained between 2×10^8 and 2×10^{10} CFUs. Preparing the product as we did, using 3 ml of Neocate, suggests that the range of bacterial counts in a 1-ml dose would be between 6.7×10^7 and 6.7×10^9 CFUs of *B. breve* BBG-001.

A record was kept of doses omitted and of sachets wasted, and was reconciled centrally against the number of unused sachets in the package after it was collected by the trial research nurses when the baby had completed the intervention.

Administration

The intervention was prescribed using the five-digit alphanumeric identifier for the pack allocated for that baby. This was written on the side of the bijou bottle during preparation and checked by the nurses before administration. A feeding syringe was used to withdraw 1 ml of supernatant, which was given to the baby.

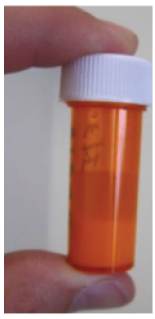


FIGURE 1 Prepared product in amber-coloured bijou bottle.

Extension of the shelf-life of the interventions: viability counts for *B. breve* BBG-001 in the active intervention

The manufacturer supplied data documenting the decline of viability and lack of contamination of previous batches of the product extending for 42 months from manufacture. As there was a lack of evidence beyond that time, the stated shelf life for the batches provided for the PiPS trial extended to the end of August 2012 for the active product and September 2012 for the placebo. During 2011 and 2012 it became clear that recruitment would need to continue until mid-2013 and that a second batch of interventions would be needed. It emerged that circumstances had changed and that new batches of intervention, particularly of placebo, could not easily be provided. We knew from work with the previous batch of the active product used for the pilot study and from monitoring undertaken in the early stages of this trial that the counts of viable *B. breve* BBG-001 were declining only slowly.

In the absence of any other guidance we accepted 2×10^8 (8.3 $\log_{10}$) CFUs of *B. breve* BBG-001 per sachet (6.7×10^7 or 7.8 $\log_{10}$-CFUs per dose) as the minimum figure that we should accept for this trial.

Analysis of unused sachets returned from centres during the early stages of the PiPS trial had been carried out and corrected to give the viable count per 1-ml dose. A plot of these data (*Figure 2*) showed a gradual decline in viable counts (solid blue line). The manufacturer provided stability data from two different batches of material for 42 months from manufacture, shown as CFUs per sachet for comparison (see *Figure 2*, solid black and green lines). All three lines are roughly parallel and extrapolation from the data obtained from the batch being used for the PiPS trial indicated that the number of viable organisms per dose would remain well above 6.7×10^7 or 7.8 $\log_{10}$-CFUs for at least 48 months (i.e. until October 2013) and probably beyond.

On the basis of these data, a successful application was made to the MHRA to extend the shelf life of both probiotic and placebo to the end of October 2013 and all sachets and boxes were relabelled accordingly. It was agreed that we should continue to monitor the counts of viable bacteria in a randomly selected unused sachet from each pack after the baby had completed its course of treatment to confirm both that the rate of decline was not accelerating and that the products remained free of contamination. It was agreed that if the viable counts fell below 2×10^8 CFUs (8.3 $\log_{10}$-CFUs) per sachet, or if any contamination was identified, recruitment would stop.

Outcomes

The rationale and definitions of outcomes are detailed in appendices 1–4 of the trial protocol, which is available on the NETSCC website.[68]

Primary outcomes

- Any baby experiencing an episode of bloodstream infection, with any organism other than a skin commensal, diagnosed on a sample of blood drawn more than 72 hours after birth and before 46 weeks' postmenstrual age, death or discharge from hospital, whichever is soonest. Skin commensals include coagulase-negative staphylococci and *Corynebacterium*.
- NEC, Bell stage 2 or 3.
- Death before discharge from hospital.

Secondary outcomes

1. Number of babies with the composite outcome of any or a combination of the three primary outcomes.

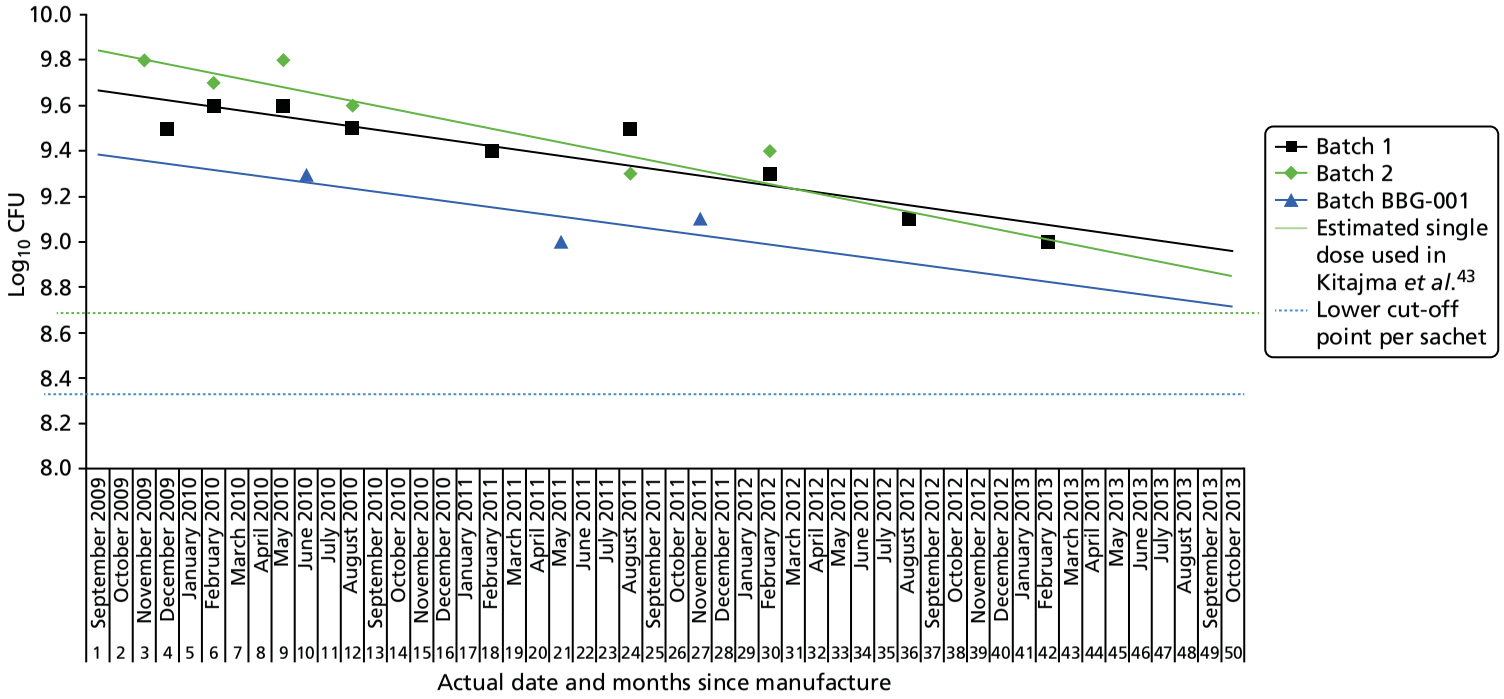


FIGURE 2 Projected decline in dosage of *B. breve* BBG-001 beyond November 2011 compared with decline in sachet content of two earlier batches supplied by the manufacturer. The *y*-axis shows the count of *B. breve* BBG-001 expressed in $\log_{10}$-CFUs. The *x*-axis shows the actual date for the trial batch of *B. breve* BBG-001 and time in months since manufacture for all batches. The solid green and black lines were provided by the manufacturer and relate to two earlier batches of the product. The counts are per sachet and extend for 42 months after manufacture. The solid blue line is the count per dose of *B. breve* BBG-001, as made up for the PiPS trial. The blue triangles represent the mean of all measurements made since the previous triangle. The regression line is extended beyond the final measurement in November 2011 to estimate the likely viability at the projected end of use of intervention in the trial in October 2013. The dashed green line at around 8.7 $\log_{10}$-CFUs represents the estimated single dose used in the trial of Kitajima *et al.*[43] The dashed blue line at 8.3 $\log_{10}$-CFUs represents the lower cut-off point per sachet of the batch of *B. breve* BBG-001 provided by the manufacturer for the PiPS trial, when prepared as for the trial this equates to 1 ml providing a dose of 6.7×10^7 or 7.8 $\log_{10}$-CFUs.

Secondary microbiological outcomes

Outcomes 2–7 are for samples taken > 72 hours after birth and before 46 weeks' postmenstrual age, death or discharge home:

2. Number of babies with any positive blood culture with an organism recognised as a skin commensal (e.g. coagulase-negative staphylococci or *Corynebacterium*).
3. Number of babies with blood cultures taken.
4. Number of blood cultures taken per baby.
5. Number of babies with episodes of bloodstream infection with organisms other than skin commensals by organism, for example *Escherichia coli*, *Klebsiella* spp., fungi, and by antibiotic resistance types, specifically meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria.
6. Number of babies with isolates of organisms other than skin commensals from a normally sterile site other than blood, for example cerebrospinal fluid, suprapubic aspiration of urine, pleural cavity, etc.
7. Number of babies with a positive culture of *B. breve* BBG-001 from any normally sterile site.

In addition:

8. Total duration of days of antibiotics and/or antifungals administered per baby after 72 hours and until 46 weeks' postmenstrual age, death or discharge from hospital, whichever is soonest, for treatment of suspected or proven late-onset sepsis, that is, excluding prophylactic use.
9. The number of babies colonised with the administered probiotic strain defined by the isolation of *B. breve* BBG-001 from stool samples at 2 weeks' postnatal age and at 36 weeks' postmenstrual age.
10. Stool flora: the number of babies colonised with MRSA, VRE or ESBL at 2 weeks' postnatal and at 36 weeks' postmenstrual age.

Nutritional and gastroenterological outcomes

11. Age at achieving full enteral nutrition (defined as 150 ml/kg/day for 1 day).
12. Change of weight z-score from birth to 36 weeks' postmenstrual age or discharge from hospital if sooner.

Other clinical outcomes

14. Bronchopulmonary dysplasia.
15. Hydrocephalus and/or intraparenchymal cysts confirmed by cerebral ultrasound scan performed during the baby's inpatient stay.
16. Worst stage of retinopathy of prematurity in either eye at discharge or death.
17. Length of stay in intensive, high-dependency and special care unit.

Randomisation

Randomisation was performed by health-care staff trained in trial procedures and named on the trial delegation log.

Randomisation to receive either probiotic or placebo used a central web-based service, with telephone back-up, based at the National Perinatal Epidemiological Unit (NPEU), University of Oxford, Oxford, UK. The randomisation program used a minimisation algorithm to ensure balance across site, sex, gestational age at birth (23, 24, 25, 26/27 and 28–30 weeks' gestation) and whether or not randomisation occurred sooner than 24 hours after birth.

At randomisation the investigator was given a unique five-digit study number for the baby, which was the principal identifier throughout the trial, and a five-digit alphanumeric number for the intervention pack to be used.

Parents, clinicians and outcome assessors were blind to the allocation.

Trial procedures

Consent

Whenever possible, preliminary discussions supported by written information about the trial would be offered to parents before the birth if the baby was likely to be eligible. This happened both at recruiting centres and at local hospitals that routinely referred babies into the recruiting centres. Informed written consent was sought from a parent after the birth only when they had been given a full oral and written explanation of the study. Copies of the parent information leaflet, the consent form and the leaflet provided to investigators summarising the material that should be covered in discussions about the trial are available in *Appendices 3–5*.

Investigators were encouraged to discuss the trial with parents periodically during the hospitalisation to confirm their continued understanding and willingness to participate.

At all stages it was made clear to the parents that they remained free to withdraw their baby from the study at any time with no need to provide an explanation. When babies were transferred between hospitals, the parents were given written information including the name of the consultant acting as principal investigator (PI) at the receiving hospital.

Parents who did not speak English were approached only if an appropriate adult interpreter was available.

Withdrawal from the trial

When parents requested that their baby be withdrawn from the trial, we completed an additional data form (see form 6, *Appendix 6*) that clarified whether or not it was only administration of the intervention that was to be discontinued and whether or not the parents were willing for data already collected, for outstanding data and/or stool collection to continue and for those data to be used.

Clinical care of participants

The day-to-day clinical care of participants was entirely at the discretion of the responsible clinical team. Investigators were encouraged to use maternal breast milk, but feeding regimes were not standardised.

Discontinuation of the trial intervention

Whether or not the intervention was discontinued temporarily when babies were unwell was at the discretion of the parents and attending clinical staff. The only circumstance in which clear advice was given to withhold a dose was when intestinal perforation was suspected.

Stool sample collection

Stool samples were collected as close as possible to 14 days' postnatal and 36 weeks' postmenstrual age. These times were chosen for practical reasons, the main objective being to gain a snapshot of stool colonisation by *B. breve* BBG-001 as a marker of intestinal colonisation. It was considered that, at 2 weeks of age, enteral feeds would be established in the majority of babies, who would have received the intervention for over a week, while still being before the time, for this population, of the peak incidence of NEC. Thirty-six weeks was selected, as this is the time at which outcome data describing bronchopulmonary dysplasia and growth were reported. Investigators were asked, if possible, to send three full scoops of stool. Samples were posted for processing to the Microbiology Laboratory at the Royal London Hospital, Barts Health NHS Trust, UK, using the Thermacor transportation system for diagnostic samples (Dyecor Ltd, Hereford, UK) and the Royal Mail.

No other biological samples were collected.

Data collection

With the exception of detailed results of routine microbiological investigations, all trial data were collected onto paper forms, which were posted to the NPEU Clinical Trials Unit for checking and double-entered onto a web-based clinical database, OpenClinica (OpenClinica, LLC, Waltham, MA, USA). Data were entered in accordance with NPEU Clinical Trials Unit OpenClinica data entry conventions. All personal details were entered into a Microsoft Access® 2013 database (version 15, Microsoft Corporation, Redmond, WA, USA).

Form 1: trial entry (see *Appendix 7*)

Part A of this form had to be completed and the answers available to facilitate randomisation and parts B–F of the form comprised baseline maternal and neonatal information. It was requested that it was posted to the trial office within 1 week of birth.

Form 2: daily data collection (see *Appendix 8*)

The aim of this form was to collect details of enteral feeds and antimicrobial interventions for the first 14 days of life until the collection of the first stool sample so as to enable later detailed analysis of determinants of colonisation at 14 days with *B. breve* BBG-001. If the baby was transferred between hospitals during this time, then a copy was retained at the referring hospital and the original form accompanied the baby.

Form 3: clinical details of baby at transfer, discharge or death (see *Appendix 9*)

This form provided clinical details and was due for completion at discharge from hospital, at death or if the baby was transferred to a different hospital, that is one form was completed for each admission and a baby could accrue multiple forms. If the baby reached 36 weeks' postmenstrual age during the admission, the details of growth and respiratory support to determine whether or not the baby had bronchopulmonary dysplasia were provided. The form included a question about whether or not the baby had experienced any episode of NEC or other abdominal pathology which, if affirmative, led to completion of form 4.

Form 4: abdominal pathology (see *Appendix 10*)

This form was completed for any episode of suspected abdominal pathology. Multiple forms, each for a different episode, might be completed during a single admission covered by a single form 3 and if a baby was transferred between hospitals for specialist management of NEC multiple forms might be received from different hospitals for the same episode. The staging of an episode of NEC was primarily based on that provided by the clinician on the form but the form included questions about the clinical characteristics of the episode with the intention that these would later be checked to confirm consistency with the stated NEC staging.

Form 4 review

All cases in which any form 4 had been received were reviewed by Professor Kate Costeloe (chief investigator), Dr Kenny McCormick (consultant neonatologist, PI for the PiPS trial at the John Radcliffe Hospital, Oxford, UK) and Mrs Michele Upton (PiPS research nurse), to determine the number of discrete episodes of NEC, the highest Bell staging of any NEC episode, the age at onset of the first episode of any NEC and of stage 2 or 3 NEC and the agreed diagnosis of episodes of other abdominal pathologies. The review involved scrutiny of all forms 4 together with the associated forms 3 and, when relevant, with postmortem reports and operation notes. Outstanding inconsistencies and queries were resolved together with the PIs with reference to the contemporaneous medical records.

Routine microbiological data

The results of routine microbiological investigations together with the antibiotic sensitivities of cultured bacteria were obtained directly from the staff in the laboratories of participating hospitals on an Microsoft Excel® 2010 spreadsheet (Version 14, Microsoft Corporation, Redmond, WA, USA). They were scrutinised individually by Dr Michael R Millar to ensure that all positive cultures from normally sterile sites were identified. All positive cultures were entered onto the trial database together with the sampling site, species of bacteria and patterns of antibiotic resistance. The accuracy of the trial microbiological data was checked by comparing 20% of trial data entries against the Microsoft Excel-recorded laboratory returns.

Safety and adverse event reporting

Unexpected serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) were reported using form 5 (see *Appendix 11*). Two SUSARs were noted prospectively:

1. intestinal obstruction associated with maize starch
2. bacteraemia with *B. breve* BBG-001.

Stool samples: microbiology methods

All samples were processed in the microbiology laboratory at Barts Health NHS Trust. When multiple samples were received from the same infant, then the sample collected closest to the appropriate date (14 days postnatal age or 36 weeks' postmenstrual age) was selected for storage and analysis.

The microbiology laboratory at Barts Health NHS Trust is accredited through Clinical Pathology Accreditation (UK) Ltd (a wholly owned subsidiary of the United Kingdom Accreditation Service). The procedures used in this study, such as those used for culture, identification, antibiotic sensitivity testing of organisms and disposal of waste, were performed in accordance with laboratory standard operating procedures.

On receipt into the laboratory, specimens were weighed and divided in to two equal parts. The study number and date of receipt of each specimen were recorded. Half was frozen and stored at –80 °C; this sample was collected to allow additional chemical, immunological and molecular analyses including the molecular detection of the trial strain (*B. breve* BBG-001). The other half was diluted 1 : 10 in a cryopreservative broth [brain–heart infusion broth (Oxoid Microbiological Products Ltd, Basingstoke, UK) containing 10% glycerol (weight/volume)], mixed by vortexing for 10 seconds, and then placed in 1-ml aliquots into sterile 1.5-ml Eppendorf tubes (Eppendorf AG, Hamburg, Germany) before freezing at –80 °C.

Detection of Bifidobacterium breve *BBG-001, meticillin-resistant* Staphylococcus aureus*, vancomycin-resistant enterococci and extended-spectrum beta-lactamase-producing Gram-negative bacteria in stool samples by culture*

Samples were batch processed. Vials of frozen sample in cryopreservative were allowed to thaw at room temperature, and 100 µl of the faecal broth was serially diluted in phosphate-buffered saline. Aliquots of 100 µl of the neat, 10^{-1}, 10^{-3} and 10^{-5} dilutions were inoculated onto the agar medium plates.

Selective media were used for the detection of the trial strain (*B. breve* BBG-001), MRSA, VRE and ESBL-producing Enterobacteriaceae. The selective medium used for detection of the *B. breve* BBG-001 was Trypticase® peptone oligosaccharide (TOS) agar (Yakult Honsha Ltd, Japan) containing carbenicillin (10 µg/ml) and streptomycin (50 µg/ml). TOS agar was incubated for 48–72 hours anaerobically. ESBL-producing Enterobacteriaceae were cultured using MacConkey agar (Oxoid Microbiological Products Ltd, Basingstoke, UK) and Brilliance™ ESBL agar (Oxoid Microbiological Products Ltd), MRSA using mannitol salt agar with oxacillin (Oxoid Microbiological Products Ltd) and VRE using Slanetz and Bartley agar with vancomycin (Oxoid Microbiological Products Ltd). Inoculated selective media for MRSA, VRE and ESBL were incubated at 37 °C for 24–36 hours in air.

Identification and enumeration of cultured Bifidobacterium breve

Bifidobacterium breve produces a characteristic white convex colony on TOS agar. The faecal concentration of *B. breve* was determined by counting the number of colonies of faecal dilutions on TOS agar, allowing estimation of the numbers in the undiluted samples. Cultured colonies were identified using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (Bruker UK Ltd, Coventry, UK). In the early phase of the study the identification of a proportion of representative colonies was confirmed by 16S ribosomal deoxyribonucleic acid (DNA) sequencing.

Identification and enumeration of antibiotic-resistant bacteria

Bacterial colonies growing on selective agars were enumerated. Isolates were identified using standard laboratory methods including MALDI-TOF mass spectrometry (Bruker UK Ltd, Coventry, UK). MRSA and VRE isolates were identified as MRSA or VRE using British Society for Antimicrobial Chemotherapy methods and interpretive criteria.[69] Gram-negative bacilli which grew on MacConkey or Brilliance ESBL agar were tested for susceptibility to a range of antibiotics using British Society for Antimicrobial Chemotherapy methods.[69] Antibiotics tested included cefuroxime, ceftazidime, cefpodoxime, ampicillin, gentamicin, piperacillin/tazobactam, amoxicillin with clavulanate, tetracycline, trimethoprim, amikacin, tobramycin, imipenem, ertapenem, tigecycline, colistin, ciprofloxacin, aztreonam and chloramphenicol. Antibiotic-resistant isolates were stored by emulsification of colonies into microbank storage vials (Pro-lab Diagnostics, Wirral, UK) and stored at –80 °C.

Molecular detection of Bifidobacterium breve *strain BBG-001 in stool samples*

Deoxyribonucleic acid was extracted from faecal matter using the QIAamp DNA stool minikit (Qiagen Ltd, Manchester, UK) in accordance with the manufacturer's instructions with an added bead-beating step. A strain-specific quantitative real-time PCR method previously reported by Fujimoto *et al.* in 2011[70] was used to detect *B. breve* BBG-001. The forward PCR primer sequence was 5′-ATGGCAAAACCGGGCTGAA-3′ and the reverse 5′-CCCACCTCTCATCCGC-3′ to give a 313-bp PCR product. Amplification and detection was carried out in 96-well optical low-profile plates (Anachem Ltd, Luton, UK) on a Bio-Rad CFX 96 real-time PCR machine-C1000 thermal cycler (Bio-Rad, Hercules, CA, USA). The PCR assay was re-optimised for use on the Bio-Rad machine with a fast PCR mix. Several PCR mixes were tested (Bio-Rad, Molzym and Agilent). The annealing temperature was optimised using the temperature gradient function of the Bio-Rad PCR machine. A primer titration was also performed.

Each PCR reaction (final/total volume of 10 µl) contained 1 µl of DNA template, 400 nM of each PCR primer, and 2 × Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technologies, Santa Clara, CA, USA). The 313-bp target sequence was amplified with an initial hold of 3 minutes at 95 °C followed by 40 cycles of 5 seconds at 95 °C and 10 seconds at the optimised annealing temperature of 62 °C. The melt cycle involved a temperature ramp from 65 °C to 95 °C, with a 5-second hold at each 0.5 °C step of the ramp.

Each sample was analysed in triplicate at neat and 1 : 10 concentration to check for PCR inhibition. A no-template control, a faecal extraction-negative control and a faecal extraction-positive control were also included on each PCR run. A sample was scored as positive if there is amplification for two or three out of three replicates with a melt curve at 87 °C, 82 °C or 85 °C, because we found that the strain-specific sequence derived from stool samples did sometimes give an 82 °C or 85 °C melt curve. Purified *B. breve* BBG-001 DNA produced a melt curve of 87 °C. Serial 10-fold dilution standards were run on each plate using the trial strain DNA at concentrations from 30 ng/µl to 30 fg/µl to allow estimation of the quantity of *B. breve* BBG-001 in each sample.

Data validation

Validation programs performed a series of range, logic and missing data checks to identify any inconsistencies within and across forms on an ongoing basis. Some queries were resolved at the NPEU according to predefined protocols; those that could not be resolved were reconciled between the staff in the trial office, the PiPS trial research nurses, chief investigator and PI with reference to the clinical record, and documented accordingly.

Sample size

Estimating sample size was difficult because of the paucity of reliable contemporary outcome data by gestational age. Based on data collected for local service appraisals around the year 2000, it was thought that the event rate of each of the primary outcomes might be as high as 15%. At a two-sided significance level of 5%, a trial of 1300 infants would have 90% power to detect a 40% relative risk (RR) reduction from 15% to 9.1%, or a 44% RR reduction from 12% to 6.7% or from 10% to 5.6% for each of the primary outcomes. These reductions were deemed to be of clinical importance by the investigators.

Recruitment targets

The aim was to begin recruitment within 6 months of trial commencement in September 2009, to recruit 300 babies in the first 3 months and accelerating gradually so that thereafter an average of 50 babies were recruited each month, with a total recruitment time of 2 years and 6 months.

Statistical methods

The full statistical analysis plan is available in *Appendix 12*.

The comparison of primary interest was whether or not there was a difference between the groups of the trial in any of the three primary outcomes. The primary analysis of primary and secondary outcomes was by intention to treat, that is the outcomes were compared across randomised groups for all infants recruited regardless of whether or not, or for how long, they received the allocated PiPS trial interventions.

Adjusted analyses were performed on all comparative analyses adjusting for the variables used in the minimisation algorithm, hospital, sex, gestational age at birth (23, 24, 25, 26/27 and 28–30 weeks' gestation), and whether or not randomisation occured sooner than 24 hours after birth. The adjusted analyses also took into account correlation of outcomes between participating babies from multiple births.

For binary outcomes, for example whether or not a baby ever had an episode of infection, adjusted RRs and CIs were calculated. For continuous outcomes, for example the number of episodes of infection, adjusted differences in means or unadjusted differences in medians (depending on the distribution of the data) were calculated with CIs. Analysis of time-to-event outcomes, such as reaching full enteral feeding, used survival analysis techniques.

Subgroup analyses included an interaction test and, when appropriate, results are presented as adjusted RRs with 95% CIs.

Prespecified subgroup analyses were performed on the primary outcomes by intention to treat, stratified by:

- whether or not randomised in the first or second 24 hours after birth
- gestational age at birth as per minimisation: 23, 24, 25, 26/27 and 28–30 weeks' gestation
- male versus female
- colonised versus not colonised at 2 weeks' postnatal age
- gestational age $< 28^{+0}$ or $\geq 28^{+0}$ weeks' gestation.

An additional subgroup analysis was performed post hoc by birthweight above or below 1 kg to facilitate comparison with data from the published ProPrems trial[41] and to address recommendations made in successive systematic reviews[30,32,35] concerning routine administration of probiotics in these birthweight categories.

A secondary analysis of all clinical and microbiological outcomes was conducted in those babies for whom stool colonisation data were available at 2 weeks' postnatal age by whether or not the baby was colonised with *B. breve* BBG-001 identified by either culture or PCR.

Determinants of successful colonisation at 2 weeks' postnatal age in those babies analysed to receive probiotics were investigated using forward stepwise regression. The factors assessed were baseline characteristics together with day of first feed, type of milk, use of antacid and administration of antibiotics beyond the fifth day after birth.

Significance levels and multiplicity

The 95% CIs are presented for all analyses on the primary outcomes, and a significance level of 5% (consistent with a 95% CI) used to indicate statistical significance.

Owing to the large number of secondary outcomes, all analyses are presented with 99% CIs and a significance level of 1% (consistent with a 99% CI) is used to indicate statistical significance.

The *p*-values are not presented for comparative analyses, but are for tests of interaction.

Regulatory approvals and protocol changes

Version 1.0 of the protocol, dated 29 January 2009, was approved by the South Central Oxford A Ethics Committee on 12 May 2009. The name of the trial was changed from PREFER to PiPS (as a condition of approval from the ethics committee) and this resulted in the protocol being amended to version 2.0, dated 18 May 2009.

Two changes to the conduct of the trial resulted in protocol version 3.0 (dated 6 October 2009): the way the investigational medicinal product (IMP) was allocated (from study number to pack number) and the duration of daily data collection of milk feeds and antibiotic/antifungal usage (from 'until full feeds reached' to 'until 2 weeks' postnatal age'). Minor typographical changes resulted in version 3.1 (dated 3 February 2010), and an update to include a more recent appraisal of the literature on *Bifidobacterium* use in infants led to version 4.0 (dated 13 April 2010).

Two sections relating to safety reporting and the addition of continuing care sites were updated in March 2010 (version 5.0, dated 17 March 2011). Clarification was made to safety reporting at different 'levels' of sites to state that safety will be assessed continuously and reported irrespective of site status. The description of how the addition and set-up of continuing care sites was achieved in practice was updated, and the implementation of the generic site-specific application system and 'statement of responsibilities' for gaining approvals of continuing care sites that fell outside recognised clinical pathways for transfers was added. All of these amendments received Research Ethics Committee approval.

A number of changes to the trial protocol resulted in version 6.0 (dated 24 July 2012). The background and rationale section of the protocol was updated with information from the latest publications, and the window for primary outcome data for late-onset sepsis and secondary outcome data collection for microbiological culture and antibiotic/antifungal use was 'closed' at 46 weeks' postmenstrual age for those babies still in hospital. A paragraph was added to appendix 6 of the protocol about the identification of carbapenem (imipenem, meropenem)-resistant Enterobacteriaceae cultures from stool samples taken from the PiPS trial participants and the procedure for alerting sites of this finding. Details of flagging in sections 3.11 and 10.6 of the protocol were modified to reflect changes in the current Medical Research Information Service system and what services could be provided under the current project remit. The lower

limit of eligibility for gestational age, which had not been clear in previous versions of the protocol, was explicitly defined. The specified dose range for the IMP was changed from between 2.2×10^9 and 3.2×10^9 CFUs to between 6.7×10^7 and 6.7×10^9 CFUs along with the expiry date for the IMP and placebo in substantial amendment number 5. References to this specification range in the protocol were all updated to reflect this change.

The final version of the protocol (version 6), dated July 2012, is available on the NETSCC website at www.nets.nihr.ac.uk. All other trial documents are listed in *Table 2*, with details of amendments and version change.

TABLE 2 Versions of trial documents other than the protocol that were in use at the end of the trial with details of amendments

Study document	Version as of April 2014	Changes
Consent form	Version 3.1, 20 January 2010	Amendments made before the start of recruitment included additional questions for flagging of babies and use of personal identifiable data. Minor amendments to wording of question on confidentiality (to be in line with National Information Governance Board for Health and Social Care recommendations), form design and instructions on how to use it
Parent information leaflet	Version 5.1, 14 February 2011	Amendments made during recruitment included minor formatting and typographical errors changes, updating of text on participant 'flagging' and consent for providing primary care trust details (in line with NHS Information Centre recommendations), and an update to the current appraisal of literature on probiotic use in infants
General practitioner letter	Version 3.0, 25 July 2012	Amended at the time of sending to change the trial name from PREFER to PiPS and to add the mother's name and date of birth
Transfer contact sheet	Version 1.0, 21 March 2010	No amendments. For parents of babies that have been transferred between hospitals. This document will give the contact details of the study team at the receiving hospital
Form 1: trial entry	Version 3.0, 20 June 2011	Amendment during recruitment. Correction of clerical errors, minor formatting of form design, and addition and clarification of instructions
Form 2: daily data collection	Version 3.0, 21 March 2011	Addition during recruitment of a question about stool collection and instructions for form completion. Correction of clerical errors and clarifications
Form 3: clinical details of baby at transfer, discharge or death	Version 4.0, 20 June 2011	Addition during recruitment of questions clarifying stool collection and the date of last dose, removal of redundant questions on respiratory support. Correction of clerical errors, minor formatting to form design and addition of instructions
Form 4: abdominal pathology	Version 3.0, 20 June 2011	Removal during recruitment of a redundant subquestion on NEC, correction of clerical errors, minor formatting to form design and addition of instructions
Form 5: SAE/SUSAR reporting	Version 2.0, 30 June 2010	Correction prior to the beginning of recruitment of clerical errors, minor formatting to form design and addition of instructions
Form 6: discontinuation or withdrawal	Version 3.0, 20 June 2011	Correction during recruitment of clerical errors, minor formatting to form design and addition of instructions

Monitoring

Central monitoring was performed throughout the trial by the NPEU Clinical Trials Unit co-ordinating centre to ensure that case report forms were complete and to detect unusual patterns and outliers in data. In addition, on-site monitoring was completed for 98% of participating sites, which involved inspection of site files and checks on compliance with trial procedures and good clinical practice (GCP). Site audits and source verification of data were carried out only if 'triggered' by central monitoring or from routine site visits undertaken by PiPS research; no such triggers occurred during the trial.

Trial oversight and patient and public involvement

The trial was overseen by an independent Trial Steering Committee and Data Monitoring Committee. The Trial Steering Committee first met during the planning stages of the trial and supported the investigators while clarification was being obtained from the MHRA around the status of probiotics and the requirement for a Clinical Trial Certificate. Thereafter, the committees met annually.

The membership of the Trial Steering Committee included a representative of the preterm baby charity Bliss who represented parents and who advised mainly on the development of the trial and the production of parent information.

The general conduct of the trial was managed by a trial co-investigators group including investigators, trial co-ordinator, research nurses, statisticians and other staff of the NPEU who met every 4–6 weeks.

Results

A total of 1315 infants were recruited from 24 hospitals within 60 miles of London (UK) over 37 months from July 2010. Details of recruitment, by site, are given in *Table 3*. The start of recruitment was delayed by 3 months so that it began 9 months after the core trial staff came into post; recruitment rates were initially slow, so that after 9 months only 121 babies had been recruited and it was 17 months before 50 babies were recruited in 1 month (*Figure 3*).

It subsequently emerged that a total of six protocol deviations concerning baseline data items had occurred at randomisation: one infant in each group was over 48 hours old and two in each group were outside the target gestational age range (*Table 4*).

There was almost complete retrieval of data entry forms (*Table 5*).

The parents of five babies withdrew consent for all participation, including for the use of data already collected (*Figure 4*).

Of the 1315 babies randomised, eight never received any intervention (seven of these died within 1 week of birth and one was transferred early to a hospital without the necessary regulatory approvals to administer the intervention). Two babies randomised to receive placebo were wrongly allocated probiotic packs.

Interim analyses

Interim analyses were undertaken when entry data for 371 babies and outcome data for death and NEC were available for 138 babies and again when entry data for 936 babies and outcome data for 598 babies were available. The results were reviewed by the Data Monitoring Committee, which recommended that recruitment should continue with no changes in target numbers.

TABLE 3 Recruitment by site

Hospital	Start date	Total recruited	Consent withdrawn for use of data, *n*	Allocated to receive probiotic, *n* (%)	Allocated to receive placebo, *n* (%)
Homerton University Hospital, London	10 June 2010	263	3	126 (19.4)	134 (20.3)
Royal London Hospital, London	21 July 2010	74	–	37 (5.7)	37 (5.6)
Whipps Cross University Hospital, London	4 August 2010	28	–	14 (2.2)	14 (2.1)
Queen's Hospital, Romford, London	20 August 2010	60	–	29 (4.5)	31 (4.7)
Newham University Hospital, London	24 September 2010	66	–	32 (4.9)	34 (5.2)
Medway Maritime Hospital, Kent	21 October 2010	76	–	40 (6.2)	36 (5.5)
St Thomas' Hospital, London	29 October 2010	97	2	46 (7.1)	49 (7.4)
North Middlesex University Hospital, London	3 December 2010	22	–	11 (1.7)	11 (1.7)
St Peter's Hospital, Chertsey	8 December 2010	96	–	46 (7.1)	50 (7.6)
William Harvey Hospital, Ashford, Kent	9 December 2010	61	–	29 (4.5)	32 (4.9)
Whittington Hospital	24 January 2011	9	–	6 (0.9)	3 (0.5)
King's College Hospital, London	11 February 2011	23	–	12 (1.9)	11 (1.7)
Southend University Hospital, Essex	11 February 2011	21	–	11 (1.7)	10 (1.5)
Barnet Hospital, London	16 February 2011	31	–	15 (2.3)	16 (2.4)
University College Hospital, London	16 February 2011	93	–	45 (6.9)	48 (7.3)
University Hospital, Lewisham, London	22 February 2011	24	–	12 (1.9)	12 (1.8)
St George's Hospital, London	4 April 2011	56	–	28 (4.3)	28 (4.2)
Croydon University Hospital, London	28 April 2011	11	–	7 (1.1)	4 (0.6)
John Radcliffe Hospital, Oxford	12 May 2011	71	–	36 (5.5)	35 (5.3)
Luton and Dunstable University Hospital, Herts	20 June 2011	32	–	16 (2.5)	16 (2.4)
Watford General Hospital	20 June 2011	27	–	14 (2.2)	13 (2.0)
Tunbridge Wells Hospital at Pembury, Kent	1 October 2011	35	–	19 (2.9)	16 (2.4)
Basildon University Hospital, London	10 November 2011	11	–	5 (0.8)	6 (0.9)
Royal Sussex County Hospital, Brighton	4 May 2012	28	–	14 (2.2)	14 (2.1)
Total		1315	5	650	660

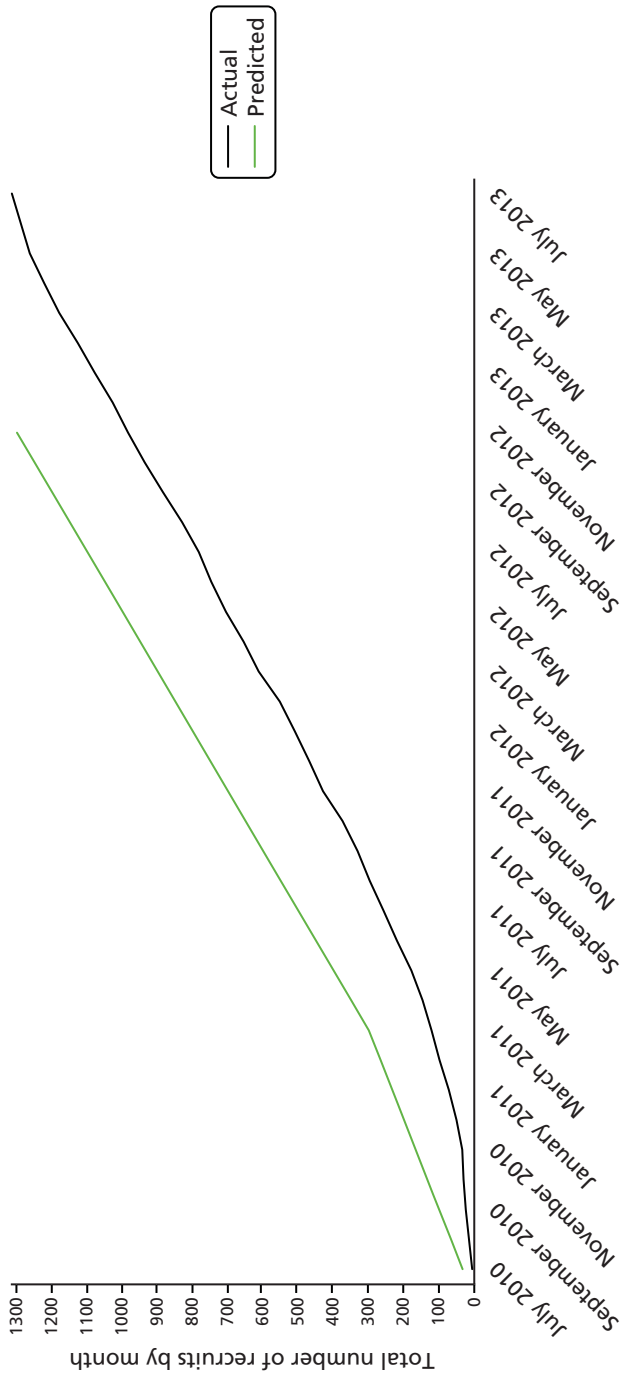


FIGURE 3 Rates of recruitment from July 2010. The target of 1294 recruits stated in the protocol was reached after 36 months but the project management group, having first confirmed with the Research Ethics Committee, extended recruitment for 1 additional month up to the IMP expiry date, in order to maximise the power of the study.

TABLE 4 Randomisation: deviations from protocol by intention to treat

Protocol deviation	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
Randomisation > 48 hours' postnatal age, *n* (%)	1 (0.2)	1 (0.2)
Gestational age < 23^{+0} weeks, *n* (%)	0	1 (0.2)
Gestational age ≥ 30^{+6} weeks, *n* (%)	2 (0.3)	1 (0.2)

TABLE 5 Data entry form retrieval rates

Form	Retrieval rate	
	Due	Received (% of total due)
Consent form	1315	1314 (99.9)
Form 1: trial entry	1315	1314 (99.9)
Form 2: daily data collection	1315	1310 (99.6)
Form 3: clinical details of baby at transfer, discharge or death	2535	2530 (99.8)
Form 4: abdominal pathology	609	609 (100)
Form 5: SAE/SUSAR	2	2 (100)
Form 6: parental discontinuation or withdrawal	37	37 (100)
Total	7128	7116 (99.8)

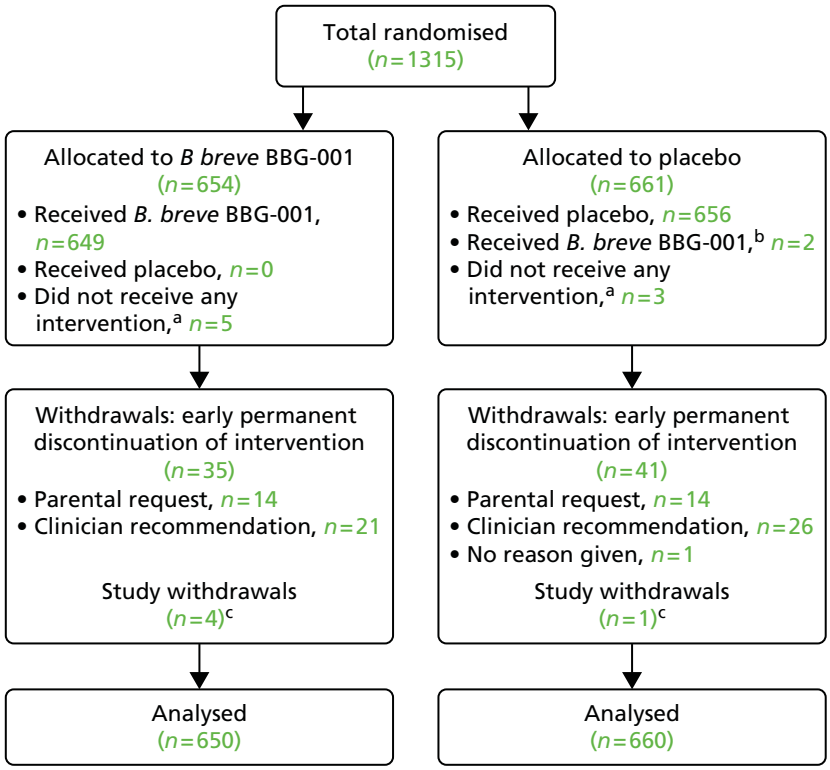


FIGURE 4 Participant flow. a, Of eight babies who received no intervention, seven died within 7 days of birth and one was transferred on the first day after birth to a hospital without the necessary approvals to administer the intervention; b, Two babies were incorrectly allocated packs containing *B. breve* BBG-001 and received that product throughout their course; c, Five sets of parents, four in the probiotic and one in the placebo group, withdrew from the trial completely and also withdrew consent for the use of data already collected.

Chapter 4 Final analysis

Baseline data

Maternal and baby characteristics were similar between the two groups (*Tables 6* and *7*). Nine per cent of babies were born outside the recruiting centre and 26% were randomised within the first 24 hours. The median age at which the first dose of intervention was administered was 44 hours.

Several of the highest recruiting hospitals are sited in multicultural inner-city areas: this is reflected in the spread of ethnicity, with 57% of babies overall being born to white women, 20% to Afro-Caribbean women and 12% to women whose families were from the Indian subcontinent.

Ninety-one per cent of the babies had been exposed to antenatal corticosteroid, 28% were born following pregnancies with rupture of the placental membranes more than 24 hours previously and 36% had been exposed to maternal antibiotics within 24 hours of birth.

The median gestational age was 28^{+0} weeks, 48% being born before 28 weeks. Mean birthweight was 1041 g, 49% being born at or below a birthweight of 1000 g.

Other early characteristics

Of 22 babies with major malformations, three in the probiotic and two in the placebo group died before discharge from hospital.

There were 1281 (98%) babies who received enteral nutrition within the first 14 days, of whom 96% received some maternal breast milk. In 48.5% of these, the maternal milk was augmented with either donor breast or formula milk (*Table 8*). Of the 29 babies who received no milk in the first 14 days, 18 died, 13 in the probiotic group and five in the placebo group. Almost all of the babies received antibiotics in the first 5 days after birth, and around 70% were given more antibiotic between day 6 and day 14. In total, 10.8% received antacid; antacid administration was recorded because of the possibility that raising the gastric pH would impact the microbiome.

Compliance

A total of 76 (5.8%) babies discontinued the intervention early, 28 at parental request and the others for clinical indications. These include a small number of babies who in the early stages of the trial discontinued the intervention early because they were transferred to a hospital that did not have the regulatory approvals to administer the intervention.

TABLE 6 Baseline data by intention to treat: maternal characteristics

Characteristic	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
***Ethnic group*, n (n/N, %)**		
White	374 (57.8)	362 (55.4)
Indian	28 (4.3)	33 (5.0)
Pakistani	17 (2.6)	20 (3.0)
Bangladeshi	32 (4.9)	30 (4.6)
Black African	96 (14.8)	100 (15.3)
Black Caribbean	32 (4.9)	31 (4.7)
Other	68 (10.5)	77 (11.8)
Missing	3	7
Maternal (years)		
Mean (SD)	30.6 (6.5)	30.9 (6.6)
Range	15–58	15–58
Missing	0	1
***Antenatal steroid use*, n (n/N, %)**		
Yes, started within 24 hours of birth	168 (26.1)	167 (25.5)
Yes, started over 24 hours before birth	412 (63.9)	440 (67.1)
None	65 (10.1)	49 (7.5)
Missing	5	4
***Membrane rupture more than 24 hours before birth*, n (n/N, %)**		
Yes	171 (27.2)	187 (29.1)
No	458 (72.8)	456 (70.9)
Missing	21	17
***Chorioamnionitis diagnosed clinically within 24 hours of birth*, n (n/N, %)**		
Yes	88 (14.4)	80 (13.0)
No	523 (85.6)	537 (87.0)
Missing	39	43
***Maternal antibiotics within 24 hours of birth*, n (n/N, %)**		
Yes	220 (36.5)	226 (36.1)
No	383 (63.5)	400 (63.9)
Missing	47	34

N = number reporting.

TABLE 7 Baseline data by intention to treat: infant characteristics

Characteristic	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
Postnatal age at randomisation (hours)		
Median	35.3	35.4
IQR	23.8–43.3	23.4–43.6
Range	0.5–50.5	0.7–48.2
< 24 hours, *n* (%)	167 (25.7)	172 (26.1)
24 to ≤ 48 hours, *n* (%)	482 (74.2)	487 (73.8)
> 48 hours, *n* (%)	1 (0.2)	1 (0.2)
Gestational age at birth (weeks)		
Median	28.0	28.0
IQR	26.1–29.4	26.1–29.6
Range	23.0–31.6	22.6–31.0
< 23 weeks, *n* (%)	0	1 (0.2)
23 to < 24 weeks, *n* (%)	20 (3.1)	17 (2.6)
24 to < 25 weeks, *n* (%)	60 (9.2)	60 (9.1)
25 to < 26 weeks, *n* (%)	69 (10.6)	73 (11.1)
26 to < 28 weeks, *n* (%)	166 (25.5)	168 (25.5)
28 to < 30 weeks, *n* (%)	217 (33.4)	219 (33.2)
≥ 30 weeks, *n* (%)	118 (18.2)	122 (18.5)
Sex		
Male, *n* (%)	374 (57.5)	370 (56.1)
Female, *n* (%)	276 (42.5)	290 (43.9)
Babies born per pregnancy		
Singleton, *n* (%)	457 (70.3)	459 (69.6)
Multiple, *n* (%)	193 (29.7)	201 (30.5)
If multiple, babies born, n *(% of multiples)*		
1	2 (1.0)	0
2	167 (86.5)	175 (87.1)
3	19 (9.8)	23 (11.4)
4	5 (2.6)	3 (1.5)
***Born in enrolling hospital,* n (n/N, %)**		
Yes	589 (90.6)	603 (91.5)
No	61 (9.4)	56 (8.5)
Missing	0	1
***Mode of delivery,* n (n/N%,)**		
Vaginal birth	309 (47.5)	310 (47.0)
Caesarean before labour onset	221 (34.0)	204 (31.0)
Caesarean after labour onset	120 (18.5)	145 (22.0)
Missing	0	1

continued

TABLE 7 Baseline data by intention to treat: infant characteristics (*continued*)

Characteristic	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
***Forceps or ventouse used,* n (n/N, %)**		
Yes	13 (2.0)	16 (2.5)
No	634 (98.0)	638 (97.6)
Missing	3	6
***Main cause of preterm birth,* n (n/N, %)**		
Prelabour rupture of membranes	184 (28.5)	182 (27.7)
Preterm labour	245 (37.9)	276 (42.0)
Antepartum haemorrhage	54 (8.4)	63 (9.6)
Pregnancy-induced hypertension	54 (8.4)	34 (5.2)
Other maternal illness	66 (10.2)	54 (8.2)
Poor fetal growth (mother well)	43 (6.7)	48 (7.3)
Missing	4	3
Birthweight (g)		
n	650	660
Mean (SD)	1039 (311.7)	1043 (317.0)
Range	450–2200	475–1935
Birthweight ≤ 1000 g, *n* (%)	317 (48.8)	327 (49.5)
Birthweight > 1000 g, *n* (%)	333 (51.2)	333 (50.5)
Birthweight z-score		
n	649	657
Mean (SD)	–0.43 (1.04)	–0.42 (1.05)
Range	–3.7 to 3.9	–3.7 to 4.1
Missing	1[a]	3[a]
***Heart rate > 100 b.p.m. 5 minutes after birth,* n (n/N, %)**		
Yes	599 (92.4)	593 (90.4)
No	49 (7.6)	63 (9.6)
Missing	2	4
***Apgar score 5 minutes after birth,* n (n/N, %)**		
0–3	25 (3.9)	15 (2.3)
4–6	86 (13.5)	96 (15.0)
7–10	524 (82.5)	531 (82.7)
Missing	15	18
***CRIB II*[71]**		
n	606	622
Mean (SD)	8.9 (3.5)	8.8 (3.4)
Range	2–20	1–19
Missing	44	38

b.p.m., beats per minute; CRIB, Clinical Risk Index for Babies; IQR, interquartile range.
a Despite complete data for gestational ages and birthweights there are four missing values for birthweight z-scores. This is because four of the babies were below the reference range of age for any given weight of –0.326 to 23 weeks.
N = number reporting, if *N* is not specified the data are complete with no missing items.

TABLE 8 Other early data collected post-randomisation by intention to treat

Characteristic	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
Age at first dose of intervention (hours)		
N	633	638
Median age (hours)	43.9	44.3
IQR	31.1–52.1	32.2–51.1
Congenital malformations[a]		
Yes, *n/N* (%)	30 (4.6)	37 (5.6)
No, *n/N* (%)	620 (95.4)	622 (94.4)
Missing, *n*	0	1
If Yes		
Minor,[b] *n* (% of congenital malformations)	19 (63.3)	24 (68.6)
Major,[b] *n* (% of congenital malformations)	11 (36.7)	11 (31.4)
Missing, *n*	0	2
Enteral feeding in the first 14 days,[c] postnatal age at first feed (days)		
Number fed within 14 days of birth	634	647
Mean age (SD)	3.2 (1.9)	3.2 (1.9)
Median age (days)	3	3
IQR	2–4	2–4
Range	1–14	1–14
Type of milk received (0–14 days)		
Any maternal breast milk, *n* (% of those fed in first 14 days)	602 (92.6)	625 (94.7)
Any donor breast milk, *n* (% of those fed in first 14 days)	131 (20.2)	139 (21.1)
Any formula, *n* (% of those fed in first 14 days)	223 (34.3)	226 (34.2)
Maternal breast milk only (0–14 days)		
Yes, *n* (%)	300 (46.2)	306 (46.4)
No, *n* (%)	350 (53.8)	354 (53.6)
Antacids and antibiotic use (0–14 days)[d]		
Any antacid given, *n* (%)	64 (9.9)	77 (11.7)
Antibiotics given in first 5 days, *n* (%)	647 (99.5)	651 (98.6)
Antibiotics given between day 6 and day 14, *n* (%)	452 (69.5)	471 (71.4)

IQR, interquartile range.

a All babies with gastrointestinal anomalies and potentially lethal anomalies of other organs systems apparent within 48 hours of birth were ineligible for recruitment. Babies with other congenital malformations becoming apparent later were followed until death or discharge from hospital.

b Malformations were classified as major only if they were life-threatening in infancy or might affect the health of the baby while on the neonatal unit in such a way as to interfere with the conduct of the trial.

c Details of early feeds were not collected if the start of feeding was deferred beyond day 14.

d Details of medications were not collected beyond day 14.

N = number reporting, if *N* is not specified, the data are complete with no missing items.

On average, infants received around 87% of the recommended doses between randomisation and 36 weeks' postmenstrual age or death if sooner (*Table 9*).

The number of those born at higher gestation age falling below the 'whisker' on *Figure 5* is largely because of babies being discharged from hospital before 36 weeks' postmenstrual age. It is thought likely that the wider range of compliance at extremely low gestation age is because of those babies having more episodes when the clinicians chose to omit doses, although the effect is only apparent in the probiotic and not the placebo group.

Quality of the interventions

No organisms other than *B. breve* BBG-001 were grown from any of the sachets returned to the laboratory at Barts Health NHS Trust.

The number of viable *B. breve* BBG-001 in the returned sachets fell as predicted during the recruitment period (*Figure 6*). The average number of viable organisms in the sachets measured in November 2013 (1 month after the final dose of intervention was given in the trial) was 1.5×10^8 CFUs with a range from 7.0×10^7 to 1.5×10^8 CFUs. The lowest count remained above the level of 6.7×10^7 CFUs that had been agreed with the MHRA to be the low threshold for dosage.

Primary outcomes by intention to treat

There was no evidence that administration of the probiotic had a beneficial effect on any of the primary outcomes. The proportion of infants who had an episode of NEC Bell stage 2 or 3 was 10.0% in the probiotic group, compared with 9.4% in the placebo group (adjusted RR 0.93, 95% CI 0.68 to 1.27); the corresponding figures for late-onset sepsis were 11.7% and 11.2% (adjusted RR 0.97, 95% CI 0.73 to 1.29) and for death were 8.5% and 8.3% (adjusted RR 0.93, 95% CI 0.67 to 1.30) (*Table 10*).

TABLE 9 Compliance with intervention by intention to treat

	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
Permanent early discontinuation, *n* (%)	35 (5.4)	41 (6.2)
Reason for permanent early discontinuation, *n* (*n*/*N*, %)		
Parental request	14 (2.2)	14 (2.1)
Clinician recommendation	21 (3.2)	26 (3.9)
Missing	0	1
Per cent recommended doses[a] taken between randomisation and 36 weeks' postmenstrual age, *n*	597	608
Mean (SD), %	86.7 (21.3)	87.8 (19.5)
Missing, %	31	34
Data unreliable and set to missing,[b] %	22	18

a The number of recommended doses is based on the number of days between randomisation and 36 weeks' postmenstrual age or death if sooner. Proportions are > 100% if more doses than recommended were given.
b If the reported duration of 'temporary' interruption of administration of the intervention was greater than the total of recommended doses we concluded that the data were unreliable and both the temporary interruption and percentage of recommended doses taken were set to missing.

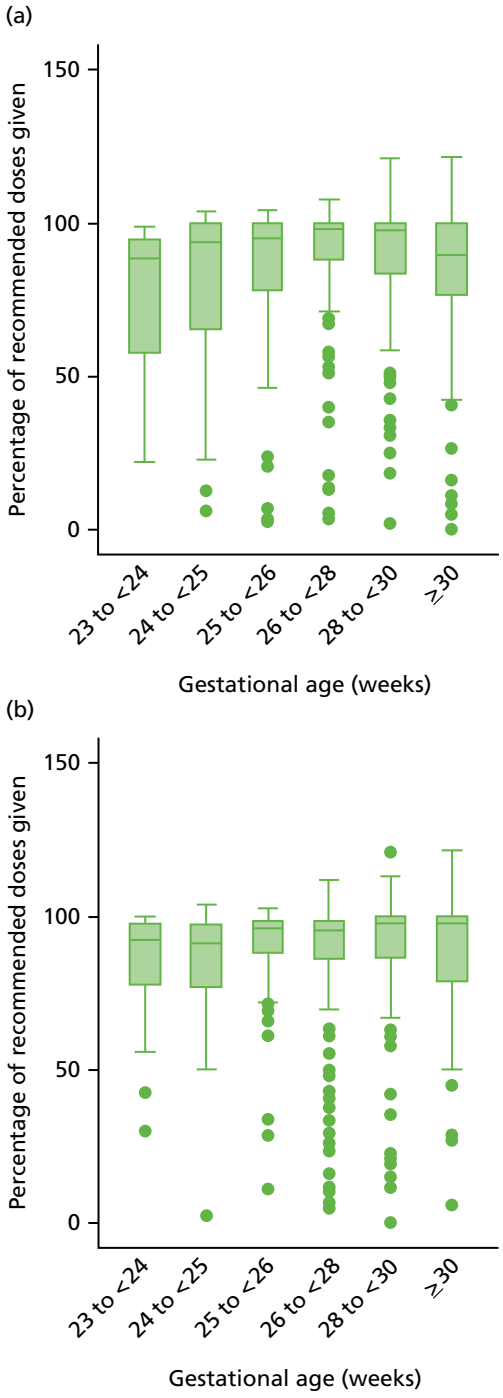


FIGURE 5 Adherence to trial intervention by allocation, gestational age and intention to treat for (a) probiotic group; and (b) placebo group. Box and whisker plot by gestational age and percentage of recommended doses that were given. The whiskers represent the adjacent values (1.5 × interquartile range).

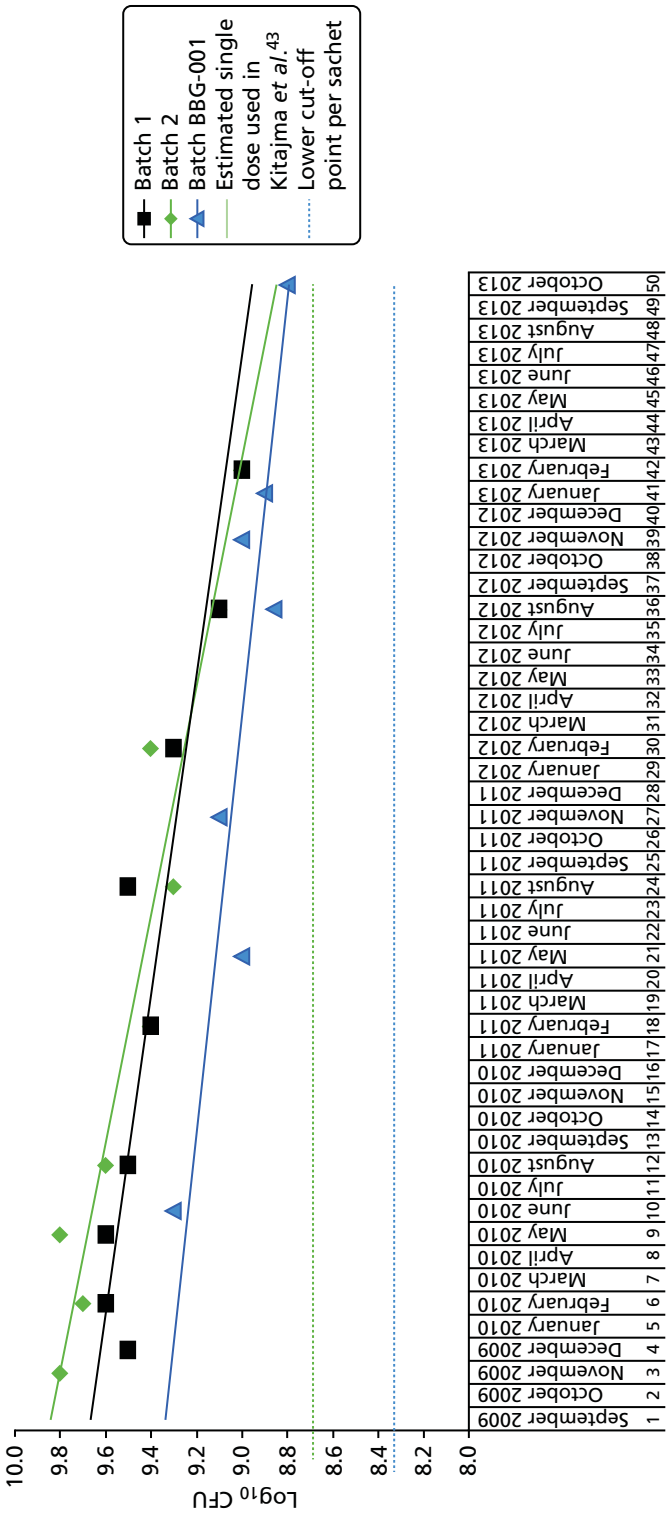


FIGURE 6 Viability of *B. breve* BBG-001 in returned sachets over the recruitment period. The *y*-axis represents the count of *B. breve* BBG-001 expressed in $\log_{10}$ CFUs. The *x*-axis represents actual date for the batch of *B. breve* BBG-001 used in the PiPS trial and time in months since manufacture for all batches. The green and black lines were provided by the manufacturer and relate to two earlier batches of the product. The counts are per sachet and extend for 42 months after manufacture. The blue line is the count per dose of *B. breve* BBG-00,1 as made up for the PiPS trial. The blue triangles represent the mean of all measurements made since the previous triangle. The continuous horizontal line at around 8.7 $\log_{10}$-CFUs represents the estimated single dose used in the trial of Kitajima *et al.*[43] The horizontal interrupted line at 8.3 $\log_{10}$-CFUs represents the lower cut-off point per sachet of the batch of *B. breve* BBG-001 provided by the manufacturer for the PiPS trial. When prepared as for the trial this equates to 1 ml providing a dose of 6.7×10^7 or 7.8 $\log_{10}$-CFUs.

TABLE 10 Primary outcomes by intention to treat

Primary analysis	Trial group		
	Probiotic ($n = 650$), n (%)	Placebo ($n = 660$), n (%)	Adjusted[a] RR (95% CI)
Late-onset sepsis[b]	73 (11.2)	77 (11.7)	0.97 (0.73 to 1.29)
NEC[c]	61 (9.4)	66 (10.0)	0.93 (0.68 to 1.27)
Death before discharge from hospital	54 (8.3)	56 (8.5)	0.93 (0.67 to 1.30)

a Adjusted for sex, gestational age at birth and randomisation within 24 hours of birth. Centre was excluded, as the model did not converge. Allowances for correlations between multiple births are accounted for.
b Late-onset sepsis is defined as bloodstream infection with non-skin commensals after 72 hours' postnatal age and before 46 weeks' postmenstrual age.
c Necrotising enterocolitis Bell stage 2 or 3.

Subgroup analyses of primary outcomes by intention to treat

The prevalence of infection associated with probiotic administration was reduced (from 7.3% to 2.8%) in the subgroup born at 28 and 29 weeks (adjusted RR 0.39, 99% CI 0.16 to 0.96). There were no other differences for the prespecified subgroup analyses for the three primary outcomes or for exploratory analyses for subgroups with birthweight > 1 kg versus < 1 kg (*Figure 7*).

Severity of necrotising enterocolitis

Prespecified exploratory analyses showed no evidence of any differences in the age at onset of NEC (median postnatal age 30 weeks in both groups) or in severity (62% of cases categorised as stage 3 in probiotic vs. 68% placebo). The primary causes of death were also similar, with 21 of 54 deaths in the probiotic group and 24 of 56 in the placebo group being attributed either to late-onset sepsis or to NEC (*Table 11*).

Secondary outcomes by intention to treat

There was also no evidence of benefit for any of the secondary outcomes including other measures of late-onset sepsis (*Table 12*); the proportion of infants with any positive blood culture after 72 hours was 28.6% in the probiotic group and 31.2% in the placebo group. The range of infecting bacteria was similar between the groups, with Enterobacteriaceae or staphylococci being identified in the majority of cases. There were three bloodstream infections attributable to antibiotic-resistant bacteria in the probiotic group and eight in the placebo group.

Infections that were not bloodstream infections were similar between the groups and there were no reports of growth of *Bifidobacterium* from any normally sterile site.

Rates of other major neonatal morbidities, administration of antimicrobials, time to establish full enteral feeds, growth up to 36 weeks' postmenstrual age and length of stay were similar between the groups (see *Table 12*).

The Kaplan–Meier plot of time to first full feed at 150 ml of milk/kg/day is a *Figure 8*.

One or both of the stool samples collected from 38 (out of 611, 6.2%) infants in the probiotic and 35 (out of 619, 5.7%) in the placebo group were colonised with antibiotic-resistant bacteria (*Table 13*).

Stool colonisation by Bifidobacterium breve *by intention to treat*

Stools were received at the microbiology laboratory at Barts Health NHS Trust from 1186 (94%) of the 1266 babies still alive at 2 weeks' postnatal age and from 1043 (83%) of the 1235 babies still alive at 36 weeks' postmenstrual age.

(a)

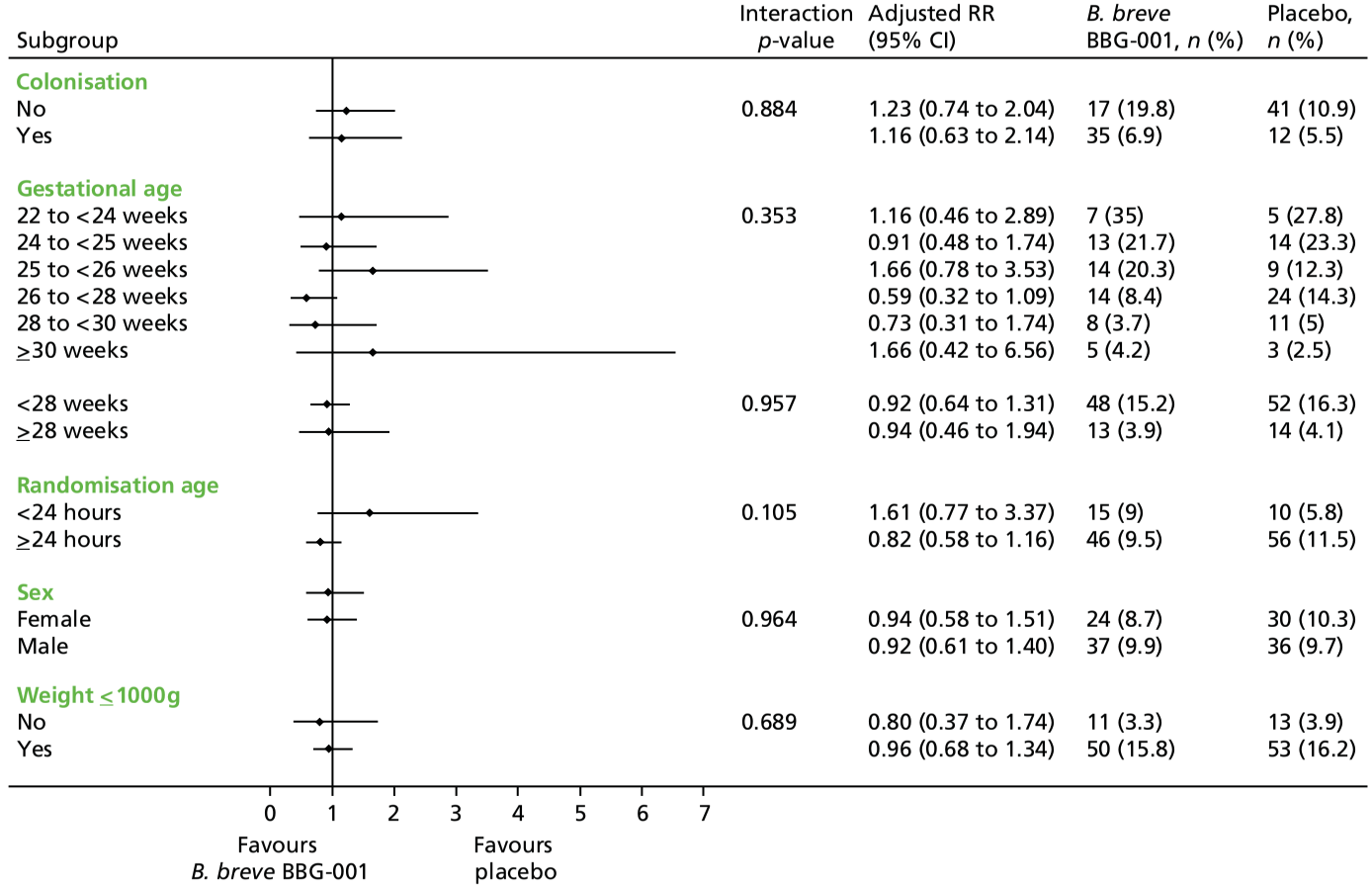
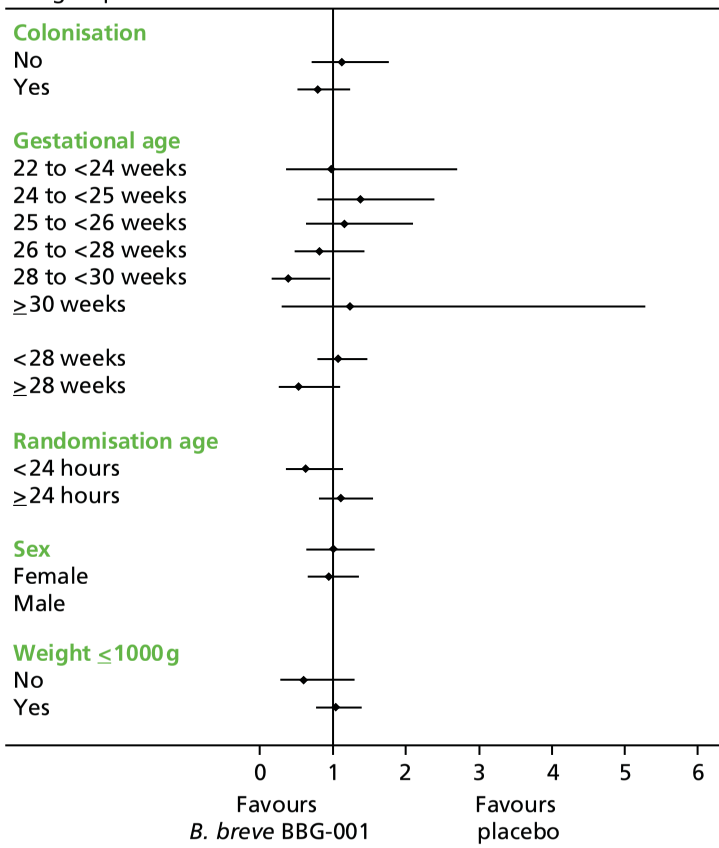


FIGURE 7 Forest plots of subgroup analyses of primary outcomes by intention to treat. (a) NEC; (b) late-onset sepsis; and (c) death at discharge. (*continued*)

(b)



Subgroup	Interaction *p*-value	Adjusted RR (95% CI)	*B. breve* BBG-001, *n* (%)	Placebo, *n* (%)
Colonisation				
No	0.303	1.12 (0.71 to 1.78)	18 (20.9)	48 (12.8)
Yes		0.80 (0.51 to 1.25)	45 (8.9)	22 (10)
Gestational age				
22 to <24 weeks	0.286	0.99 (0.36 to 2.70)	6 (30)	5 (27.8)
24 to <25 weeks		1.38 (0.79 to 2.41)	21 (35)	15 (25)
25 to <26 weeks		1.15 (0.63 to 2.11)	17 (24.6)	15 (20.5)
26 to <28 weeks		0.82 (0.47 to 1.44)	19 (11.4)	23 (13.7)
28 to <30 weeks		0.39 (0.16 to 0.96)	6 (2.8)	16 (7.3)
≥30 weeks		1.24 (0.29 to 5.29)	4 (3.4)	3 (2.5)
<28 weeks	0.092	1.07 (0.78 to 1.47)	63 (20)	58 (18.2)
≥28 weeks		0.54 (0.26 to 1.12)	10 (3)	19 (5.6)
Randomisation age				
<24 hours	0.106	0.63 (0.35 to 1.15)	14 (8.4)	24 (14)
≥24 hours		1.11 (0.80 to 1.55)	59 (12.2)	53 (10.9)
Sex				
Female	0.827	1.01 (0.64 to 1.59)	27 (9.8)	32 (11)
Male		0.94 (0.65 to 1.36)	46 (12.3)	45 (12.2)
Weight ≤1000 g				
No	0.203	0.61 (0.29 to 1.30)	10 (3)	16 (4.8)
Yes		1.04 (0.76 to 1.41)	63 (19.9)	61 (18.7)

FIGURE 7 Forest plots of subgroup analyses of primary outcomes by intention to treat. (a) NEC; (b) late-onset sepsis; and (c) death at discharge. (*continued*)

(c)

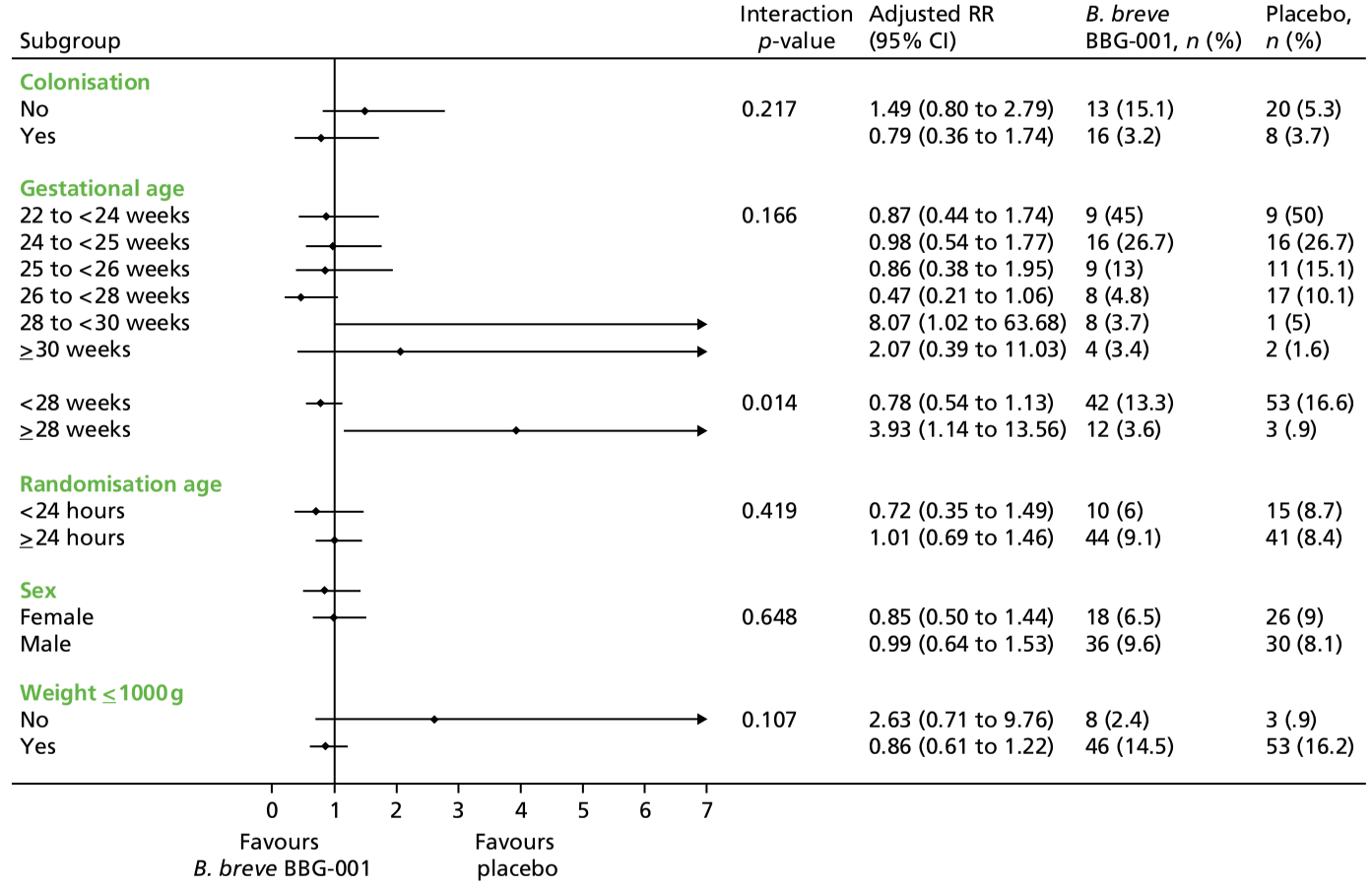


FIGURE 7 Forest plots of subgroup analyses of primary outcomes by intention to treat. (a) NEC; (b) late-onset sepsis; and (c) death at discharge.

TABLE 11 Prespecified exploratory analyses of NEC, spontaneous intestinal perforation and primary cause of death

Outcome	Trial group		
	Probiotic ($n = 650$), *n* (%)	Placebo ($n = 660$), *n* (%)	Adjusted[a] RR (99% CI)
NEC Bell stage 3	38 (5.9)	45 (6.8)	0.85 (0.51 to 1.41)
Surgery for NEC stage ≥ 2	35 (5.4)	39 (5.9)	0.89 (0.53 to 1.51)
Death attributed to NEC stage ≥ 2	9 (1.4)	14 (2.1)	0.62 (0.21 to 1.84)
Postmenstrual age (weeks) at onset of NEC stage ≥ 2, median (IQR)	30.0 (27.9 to 32.6)	30.1 (28.0 to 32.1)	–0.71 (–2.4 to 1.0)
Spontaneous intestinal perforation	7 (1.1)	4 (0.6)	1.30 (0.71 to 2.3)
Deaths attributed to infection, *n* (%)	12 (1.8)	10 (1.5)	1.10 (0.66 to 1.80)

IQR, interquartile range.
a Adjusted for sex, gestational age at birth and randomisation within 24 hours of birth. Centre was excluded, as the model did not converge. Allowances for correlations between multiple births are accounted for.

TABLE 12 Secondary outcomes by intention to treat

Secondary outcomes	Trial group		
	Probiotic ($n = 650$), *n* (%)	Placebo ($n = 660$), *n* (%)	Adjusted[a] RR (99% CI)
Late-onset sepsis[b], NEC[c] or death at discharge home	143 (22.0)	147 (22.3)	0.99 (0.79 to 1.25)
Late-onset sepsis-related and microbiological outcomes			
Positive blood culture for skin commensal	141 (21.7)	161 (24.4)	0.88 (0.69 to 1.13)
Any blood culture taken after 72 hours	490 (75.4)	519 (78.6)	0.97 (0.92 to 1.02)
Number of blood cultures per infant after 72 hours, median (IQR)	2 (1 to 4)	2 (1 to 5)	0 (0–0)
Bloodstream infection by organism			
Enterobacteriaceae	23 (3.5)	29 (4.4)	0.80 (0.41 to 1.59)
Enterococcus	13 (2.0)	14 (2.1)	0.92 (0.35 to 2.43)
Staphylococcus	21 (3.2)	17 (2.6)	1.26 (0.56 to 2.82)
Fungi	5 (0.8)	5 (0.8)	1.00 (0.20 to 5.06)
Other non-skin commensals	22 (3.4)	22 (3.3)	0.93 (0.44 to 1.96)
Antibiotic-resistant bloodstream infection			
MRSA	0	3 (0.5)	Too few data
VRE	1 (0.2)	0	Too few data
ESBL-producing Gram-negative bacteria	1 (0.2)	5 (0.8)	Too few data
Gentamicin resistant	1 (0.2)	0	Too few data

continued

TABLE 12 Secondary outcomes by intention to treat (*continued*)

Secondary outcomes	Trial group		
	Probiotic ($n = 650$), *n* (%)	Placebo ($n = 660$), *n* (%)	Adjusted[a] RR (99% CI)
Isolates of organisms from other normally sterile sites			
Suprapubic urine	1 (0.2)	1 (0.2)	Too few data
Cerebrospinal fluid	5 (0.8)	6 (0.9)	0.83 (0.18 to 3.80)
Pleural cavity	1 (0.2)	0	Too few data
Peritoneum	13 (2.0)	10 (1.5)	1.31 (0.45 to 3.84)
Other (joint fluid)	0	1 (0.2)	Too few data
B. breve BBG-001 from any normally sterile site	0	0	Too few data
Total days of antibiotics after 72 hours, median (IQR) [range]	10 (4 to 23) [0 to130]	11 (4–24) [0 to 202]	0 (–2 to 1)
Total days of antifungals after 72 hours, median (IQR) [range]	0 (0 to 0) [0 to 154]	0 (0 to 0) [0 to 79]	0 (0 to 0)
Enteral feeding and growth			
n	649	660	
Reached full feeds, *n* (%)	613 (94.5)	619 (93.8)	0.91 (0.79 to 1.06)[d]
Died before reaching full feeds, *n* (%)	32 (4.9)	37 (5.6)	
Missing, *n*	1	0	
Postnatal age at first full feed, (150 ml of milk/kg/day), days			
Median	14	14	
99% CI	13 to 16	13 to 16	
IQR	10 to 22	10 to 22	
Change in weight z-score (from baseline to 36 weeks' postmenstrual age)			
n	648	657	
Mean (SD)	–1.33 (0.93)	–1.38 (0.92)	0.03 (–0.08 to 0.15)
Range	–4.62 to 1.8	–6.76 to 2.69	
Other morbidities			
Survivors to 36 weeks' postmenstrual age, *n*	595	604	
Bronchopulmonary dysplasia (any O_2 at 36 weeks' postmenstrual age), *n* (*n*/*N*, %)	239 (40.2)	223 (36.9)	1.02 (0.87 to 1.20)
Severe bronchopulmonary dysplasia at 36 weeks' postmenstrual age[e]	87 (14.6)	73 (12.1)	1.13 (0.80 to 1.60)
n	646	657	
Hydrocephalus and/or porencephaly and/or periventricular leucomalacia noted at any time[f]	46 (7.1)	37 (5.6)	1.23 (0.72 to 2.12)
n	600	605	
Worst stage of retinopathy of prematurity ≥ stage 3 in either eye	23 (3.8)	25 (4.1)	0.91 (0.44 to 1.88)

TABLE 12 Secondary outcomes by intention to treat (*continued*)

Secondary outcomes	Trial group		
	Probiotic ($n = 650$), *n* (%)	Placebo ($n = 660$), *n* (%)	Adjusted[a] RR (99% CI)
Length of stay			
n	647	657	
Total length of hospital stay (days), median (IQR)	68 (48 to 98)	66 (46 to 95)	1 (–4 to 6)
n	649	658	
Intensive care stay (days), median (IQR)	10 (5 to 32)	12 (5 to 32)	0 (–1 to 1)
High-dependency unit stay (days), median (IQR)	20 (6 to 34)	17 (5 to 33)	1 (–1 to 3)

IQR, interquartile range.
a Adjusted for sex, gestational age at birth and randomisation within 24 hours of birth (except for fungi, urine and cerebrospinal fluid, which are not adjusted for gestational age). Centre was excluded as the model did not converge. Allowances for correlations between multiple births are accounted for.
b Late-onset sepsis is defined as bloodstream infection with non-skin commensals after 72 hours' postnatal age and before 46 weeks' postmenstrual age.
c Necrotising enterocolitis Bell stage 2 or 3.
d Hazard ratio.
e Severe bronchopulmonary dysplasia was defined as still receiving mechanical ventilatory support or, if breathing spontaneously, still receiving more than 0.1 l/minute low-flow oxygen or 30% or higher supplementary oxygen.[72]
f An additional 35 babies (11 in the probiotic group and 24 in the placebo group) had reports of haemorrhagic parenchymal infarcts but never progressed to have reports of hydrocephalus, porencephaly or periventricular leucomalacia, of these nine in the probiotic and 14 in the placebo group died before discharge from hospital.

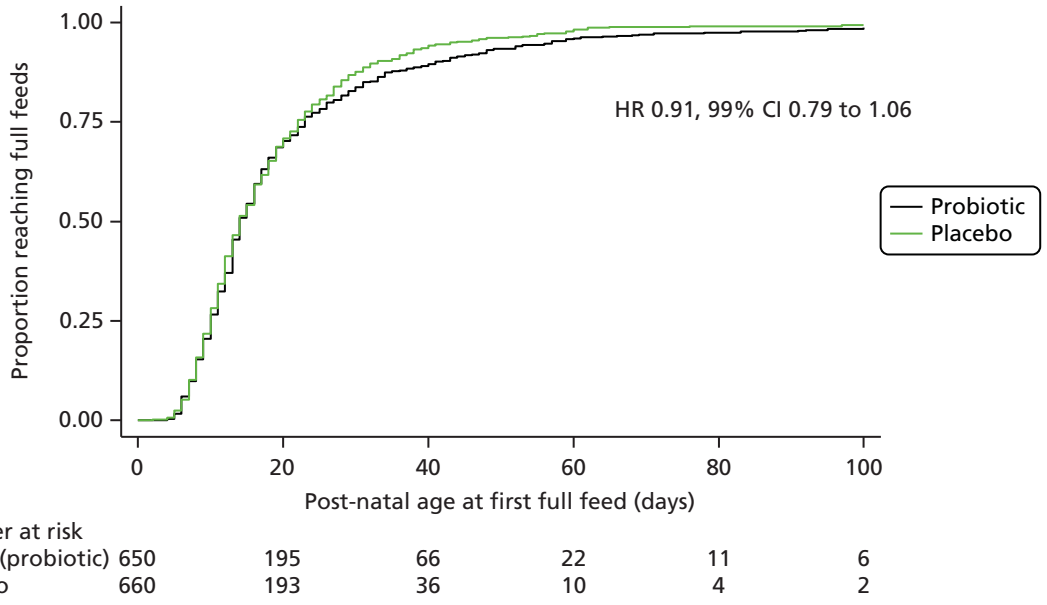


FIGURE 8 Kaplan–Meier plot of time to first full feed at 150 ml of milk/kg/day by intention to treat. HR, hazard ratio.

TABLE 13 Secondary outcomes by intention to treat: stool microbiology

Secondary outcome	Trial group		
	Probiotic ($n = 650$), n (%)	Placebo ($n = 660$), n (%)	Adjusted[a] RR (99% CI)
Stool culture at 2 weeks' postnatal age, 1266 infants alive, 1186 (94%) stool samples received			
B. breve BBG-001, n (% of received)	436 (73.8)	122 (20.5)	3.51 (2.83 to 4.34)
MRSA	1 (0.2)	2 (0.3)	Too few data
VRE	0	1 (0.2)	Too few data
ESBL-producing Gram-negative bacteria	18 (3.0)	20 (3.4)	0.76 (0.36 to 1.61)
Stool PCR at 2 weeks' postnatal age			
PCR positive, n (% of tested)	416 (84)	177 (35)	2.42 (2.06 to 2.85)
B. breve BBG-001 positive by culture or PCR	505 (85)	219 (37)	2.30 (1.99 to 2.66)
Stool culture at 36 weeks' postmenstrual age, 1245 infants alive, 1043 (84%) stool samples received			
B. breve BBG-001, n (% of received)	438 (83.6)	253 (48.7)	1.69 (1.50 to 1.91)
MRSA	1 (0.2)	0	Too few data
VRE	3 (0.6)	1 (0.2)	2.97 (0.15 to 57.67)
ESBL-producing Gram-negative bacteria	19 (3.6)	18 (3.5)	0.98 (0.44 to 2.18)

a Adjusted for sex, gestational age at birth and randomisation within 24 hours of birth. Centre was excluded, as the model did not converge. Allowances for correlations between multiple births are accounted for.

Note
Of the 1186 stools received at 2 weeks' postnatal age there was sufficient material available to perform PCR to detect *B. breve* BBG-001 on 1007.

By 2 weeks' postnatal age, *B. breve* BBG-001 was detected by culture in 73.8% of the stool samples available from babies in the probiotic group and in 20.5% of those from the placebo group, rising to 83.6% and 48.7%, respectively, at 36 weeks' postnatal age (see *Table 13*).

At 2 weeks' postnatal age, 1007 samples contained sufficient stool to also test for the presence of *B. breve* BBG-001 using PCR. Of the 123 samples from babies in the probiotic group that were culture negative, 69 (56.1%) were positive by PCR, and of the 405 negative samples in the placebo group 94 (24.0%) were positive. A total of 85% of babies in the probiotic and 37% in the placebo group had *B. breve* BBG-001 detected in their stools using either culture or PCR (see *Table 13*).

Of the 504 babies in the probiotic group who had stools cultured at both 2 weeks' postnatal and 36 weeks' postmenstrual age, 54 (10.7%) were positive for *B. breve* BBG-001 on only the first occasion, 104 (20.6%) were positive only on the second and 317 (62.9%) on both occasions. In the placebo group, 23 of 495 (4.6%) were positive on only the first occasion, 169 (34.1%) on only the second and 75 (15.2%) on both occasions.

Total colonisation rates by culture were monitored by site during recruitment and ranged at 2 weeks' postnatal age from 37.5% to 80.3% (*Table 14*). During the course of the trial those hospitals with higher colonisation rates were often those with smaller numbers of babies and at no time were there hospitals that were clearly high outliers so as to trigger additional training from the trial research nurses.

TABLE 14 Total number of babies with *B. breve* BBG-001 detected in stool either by culture or PCR at 2 weeks' postnatal age by hospital site where the baby was recruited

	Colonisation with *B. breve* BBG-001, *n* (% at site)	
Enrolling centre	Yes (*n* = 724)	No (*n* = 462)
Homerton University Hospital	142 (63.7)	81 (36.3)
University College Hospital	51 (57.3)	38 (42.7)
St Peter's Hospital, Chertsey	54 (60.7)	35 (39.3)
St Thomas' Hospital, London	46 (54.8)	38 (45.2)
John Radcliffe Hospital	34 (50.0)	34 (50.0)
Medway Maritime Hospital	49 (68.1)	23 (31.9)
The Royal London Hospital	35 (54.7)	29 (45.3)
St George's Hospital, London	35 (66.0)	18 (34.0)
Newham University Hospital	49 (80.3)	12 (19.7)
William Harvey Hospital, Ashford	33 (58.9)	23 (41.1)
Royal Sussex County Hospital	9 (37.5)	15 (62.5)
Queen's Hospital, Romford	28 (57.1)	21 (42.9)
Tunbridge Wells Hospital at Pembury	19 (57.6)	14 (42.4)
Luton and Dunstable Hospital	17 (54.8)	14 (45.2)
Watford General Hospital	17 (63.0)	10 (37.0)
Barnet Hospital	18 (62.1)	11 (37.9)
University Hospital Lewisham	14 (66.7)	7 (33.3)
Whipps Cross Hospital	18 (72.0)	7 (28.0)
King's College Hospital	15 (68.2)	7 (31.8)
Southend University Hospital	11 (61.1)	7 (38.9)
North Middlesex University Hospital	12 (70.6)	5 (29.4)
Basildon Hospital	8 (72.7)	3 (27.3)
Croydon University Hospital	5 (45.5)	6 (54.6)
Whittington Hospital	5 (55.6)	4 (44.4)

Determinants of colonisation

The results of the analysis of determinants of colonisation at 2 weeks in the probiotic group are presented in *Table 15*. In the final model, only increasing gestational age in weeks was statistically significantly associated with increased colonisation (OR 1.36; $p < 0.0001$), and the administration of any antibiotic beyond the fifth day after birth was significantly associated with less colonisation (OR 0.26; $p = 0.027$).

Administration to the mother of antibiotic in the 24 hours preceding birth was marginally associated with an increased OR (1.76; $p = 0.0506$) and increasing age in days at the start of enteral milk feeds with decreased colonisation (OR 0.90; $p = 0.0725$).

Secondary analysis by colonisation with Bifidobacterium breve *at 2 weeks' postnatal age*

At 2 weeks' postnatal age, stool colonisation data were available for 1186 babies in 724 (61.0%), of whom *B. breve* BBG-001 was detected either by culture or by PCR.

TABLE 15 Significance of determinants of successful colonisation with *B. breve* BBG-001 at 2 weeks' postnatal age for babies who were randomised to and given probiotic from whom stool samples were received ($n = 591$)

Characteristic	Single factor models	Model 2	Model 3	Final model
Postnatal age at first dose (hours)	OR 0.99 ($p = 0.0659$)			
Gestational age at birth (weeks)	OR 1.42 ($p < 0.0001$[a])	OR 1.33 ($p < 0.0001$)	OR 1.40 ($p < 0.0001$)	OR 1.36 ($p < 0.0001$)
Birthweight (kg)	OR 1.00[b] ($p < 0.0001$)			
Apgar score at 5 minutes	OR 1.15 ($p = 0.0122$)			
Day of first feed	OR 0.81 ($p < 0.0001$)			OR 0.90 ($p = 0.0725$)
Sex (male, female)	OR 1.40 ($p = 0.1614$)			
Multiple birth (single/multiple)	OR 0.98 ($p = 0.9209$)			
Ethnic group	$p = 0.4881$			
White vs. Indian	OR 0.73			
White vs. Pakistani	OR 2.91			
White vs. Bangladeshi	OR 2.19			
White vs. black African	OR 0.81			
White vs. black Caribbean	OR 1.18			
White vs. other	OR 2.00			
Antenatal steroids prior to birth (yes/no)	OR 1.00 ($p = 0.9913$)			
Membrane rupture > 24 hours before birth (yes/no)	OR 1.16 ($p = 0.5808$)			
Antibiotics in 24 hours before birth (yes/no)	OR 1.44 ($p = 0.1635$)		OR 1.94 ($p = 0.0199$)	OR 1.76 ($p = 0.0506$)
Chorioamnionitis in 24 hours before birth (yes/no)	OR 0.80 ($p = 0.5010$)			
Delivery mode (caesarean/vaginal delivery)	OR 0.62 ($p = 0.0434$)			
Formula and breast milk (day 1–14)	OR 2.21 ($p = 0.0056$)			
Formula only (day 1–14)	OR 1.29 ($p = 0.7415$)			
Breast milk only (day 1–14)	OR 0.52 ($p = 0.0151$)			
Antacids (day 1–14)	OR 1.24 ($p = 0.6096$)			
Antibiotics (day 6–14)	OR 0.14 ($p < 0.0001$)	OR 0.21 ($p = 0.0004$)	OR 0.24 ($p = 0.0014$)	OR 0.26 ($p = 0.0027$)

Variables were added to the model taking the variable with the lowest *p*-value first. All variables were retested, one by one, in the new model and the next variable was selected using the new lowest *p*-value. The final model was reached when no remaining potential variable had a *p*-value of < 0.1 and no existing variable had a *p*-value of > 0.2.
a Lowest *p*-value.
b > 1.00.

Baseline data by colonisation

The baseline characteristics of mothers and babies, together with early clinical data collected after randomisation by colonisation status at 2 weeks are presented in *Tables 16–18* and show few differences between the groups other than an under-representation among the colonised babies of those who had received only maternal breast milk and of those who had received any antibiotic after the fifth day of life.

TABLE 16 Baseline data by colonisation status at 2 weeks' postnatal age: maternal characteristics

Characteristic	Colonisation with *B. breve* BBG-001	
	Yes ($n = 724$)	No ($n = 462$)
Ethnic group		
White, *n* ($\%_{col}$, $\%_{row}$)	407 (56.5, 60.1)	270 (58.7, 39.9)
Indian, *n* ($\%_{col}$, $\%_{row}$)	31 (4.3, 57.4)	23 (5.0, 42.6)
Pakistani, *n* ($\%_{col}$, $\%_{row}$)	26 (3.6, 81.3)	6 (1.3, 18.8)
Bangladeshi, *n* ($\%_{col}$, $\%_{row}$)	38 (5.3, 70.4)	16 (3.5, 29.6)
Black African, *n* ($\%_{col}$, $\%_{row}$)	99 (13.8, 55.9)	78 (17.0, 44.1)
Black Caribbean, *n* ($\%_{col}$, $\%_{row}$)	39 (5.4, 68.4)	18 (3.9, 31.6)
Other, *n* ($\%_{col}$, $\%_{row}$)	80 (11.1, 62.0)	49 (10.7, 38.0)
Missing, *n*	4	2
Mother's age (years)		
n	724	461
Mean (SD)	30.7 (6.5)	31.1 (6.62)
Minimum to maximum	15 to 58	16 to 58
Missing	0	1
Antenatal steroid use		
Yes, started within 24 hours of birth, *n* ($\%_{col}$, $\%_{row}$)	177 (24.6, 57.8)	129 (28.2, 42.2)
Yes, started over 24 hours before birth, *n* ($\%_{col}$, $\%_{row}$)	476 (66.2, 61.6)	297 (64.9, 38.4)
None, *n* ($\%_{col}$, $\%_{row}$)	66 (9.2, 67.4)	32 (7.0, 32.7)
Missing, *n*	5	4
Membrane rupture more than 24 hours before birth		
Yes, *n* ($\%_{col}$, $\%_{row}$)	193 (27.4, 59.2)	133 (29.8, 40.8)
No, *n* ($\%_{col}$, $\%_{row}$)	511 (72.6, 61.9)	314 (70.3, 38.1)
Missing, *n*	20	15
Chorioamnionitis diagnosed clinically within 24 hours of birth		
Yes, *n* ($\%_{col}$, $\%_{row}$)	87 (12.9, 58.0)	63 (14.6, 42.0)
No, *n* ($\%_{col}$, $\%_{row}$)	588 (87.1, 61.4)	370 (85.5, 38.6)
Missing, *n*	49	29
Antibiotics in 24 hours before birth		
Yes, *n* ($\%_{col}$, $\%_{row}$)	251 (37.1, 60.9)	161 (36.7, 39.1)
No, *n* ($\%_{col}$, $\%_{row}$)	426 (62.9, 60.5)	278 (63.3, 39.5)
Missing, *n*	47	23

TABLE 17 Baseline data by colonisation status at 2 weeks' postnatal age: infant characteristics

Characteristic	Colonisation with *B. breve* BBG-001	
	Yes ($n = 724$)	No ($n = 462$)
Postnatal age at randomisation (hours)		
n	724	462
Median	35.2	35.0
IQR	22.9 to 43.5	24.0 to 43.7
Range	0.5 to 48.0	1.0 to 48.2
≤ 24 hours, *n* ($\%_{col}$, $\%_{row}$)	201 (27.8, 63.6)	115 (24.9, 36.4)
24 to < 48 hours, *n* ($\%_{col}$, $\%_{row}$)	523 (72.2, 60.2)	346 (74.9, 39.8)
> 48 hours, *n* ($\%_{col}$, $\%_{row}$)	0	1 (0.2, 100)
Gestational age at birth (weeks)		
n	724	462
Median	28.4	27.6
IQR	26.7 to 29.7	25.7 to 29.1
Range	23 to 31.6	22.6 to 30.9
< 23 weeks, *n* ($\%_{col}$, $\%_{row}$)	0	1 (0.2, 100)
23 to < 24 weeks, *n* ($\%_{col}$, $\%_{row}$)	13 (1.8, 43.3)	17 (3.7, 56.7)
24 to < 25 weeks, *n* ($\%_{col}$, $\%_{row}$)	47 (6.5, 48.0)	51 (11.0, 52.0)
25 to < 26 weeks, *n* ($\%_{col}$, $\%_{row}$)	70 (9.7, 54.3)	59 (12.8, 45.7)
26 to < 28 weeks, *n* ($\%_{col}$, $\%_{row}$)	172 (23.8, 57.7)	126 (27.3, 42.3)
28 to < 30 weeks, *n* ($\%_{col}$, $\%_{row}$)	272 (37.6, 66.7)	136 (29.4, 33.3)
≥ 30 weeks, *n* ($\%_{col}$, $\%_{row}$)	150 (20.7, 67.6)	72 (15.6, 32.4)
Sex		
Male, *n* ($\%_{col}$, $\%_{row}$)	401 (55.4, 59.9)	268 (58.0, 40.1)
Female, *n* ($\%_{col}$, $\%_{row}$)	323 (44.6, 62.5)	194 (42.0, 37.5)
Babies born per pregnancy		
Singleton, *n* ($\%_{col}$, $\%_{row}$)	496 (68.5, 59.5)	338 (73.2, 40.5)
Multiple, *n* ($\%_{col}$, $\%_{row}$)	228 (31.5, 64.8)	124 (26.8, 35.2)
If multiple, babies born, n ($\%_{col}$, $\%_{row}$)		
1	2 (0.9, 100)	0
2	194 (85.1, 64.5)	107 (86.3, 35.6)
3	26 (11.4, 63.4)	15 (12.1, 36.6)
4	6 (2.6, 75.0)	2 (1.6, 25.0)
***Born in enrolling hospital,* n ($\%_{col}$, $\%_{row}$)**		
Yes	667 (92.1, 61.3)	421 (91.1, 38.7)
No	57 (7.9, 58.2)	41 (8.9, 41.8)

TABLE 17 Baseline data by colonisation status at 2 weeks' postnatal age: infant characteristics (*continued*)

Characteristic	Colonisation with *B. breve* BBG-001	
	Yes ($n = 724$)	No ($n = 462$)
Mode of delivery		
Vaginal birth, *n* ($\%_{col}$, $\%_{row}$)	332 (45.9, 60.4)	218 (47.3, 39.6)
Caesarean before labour onset, *n* ($\%_{col}$, $\%_{row}$)	240 (33.2, 62.8)	142 (30.8, 37.2)
Caesarean after labour onset, *n* ($\%_{col}$, $\%_{row}$)	152 (21.0, 60.1)	101 (21.9, 39.9)
Missing, *n*	0	1
Forceps or ventouse used		
Yes, *n* ($\%_{col}$, $\%_{row}$)	18 (2.5, 66.7)	9 (2.0, 33.3)
No, *n* ($\%_{col}$, $\%_{row}$)	704 (97.5, 61.1)	448 (98.0, 38.9)
Missing, *n*	2	5
Main cause of preterm birth		
Prelabour rupture of membranes, *n* ($\%_{col}$, $\%_{row}$)	211 (29.3, 62.2)	128 (27.8, 37.8)
Preterm labour, *n* ($\%_{col}$, $\%_{row}$)	275 (38.2, 59.4)	188 (40.9, 40.6)
Antepartum haemorrhage, *n* ($\%_{col}$, $\%_{row}$)	69 (9.6, 63.9)	39 (8.5, 36.1)
Pregnancy-induced hypertension, *n* ($\%_{col}$, $\%_{row}$)	51 (7.1, 67.1)	25 (5.4, 32.9)
Other maternal illness, *n* ($\%_{col}$, $\%_{row}$)	68 (9.4, 62.4)	41 (8.9, 37.6)
Poor fetal growth (mother well), *n* ($\%_{col}$, $\%_{row}$)	46 (6.4, 54.1)	39 (8.5, 45.9)
Missing, *n*	4	2
Birthweight (g)		
n	724	462
Mean (SD)	1084 (312.4)	1000 (304.9)
Range	450 to 1935	475 to 1845
Birthweight ≤ 1000 g, *n* ($\%_{col}$, $\%_{row}$)	309 (42.7, 55.0)	253 (54.8, 45.0)
Birthweight > 1000 g, *n* ($\%_{col}$, $\%_{row}$)	415 (57.3, 66.5)	209 (45.2, 33.5)
Birthweight z-score[a]		
n	722	460
Mean (SD)	–0.39 (1.02)	–0.44 (1.09)
Range	–3.69 to 4.09	–3.65 to 3.92
Missing	2	2
Heart rate > 100 b.p.m. 5 minutes after birth		
Yes, *n* ($\%_{col}$, $\%_{row}$)	669 (92.5, 61.7)	415 (90.6, 38.3)
No, *n* ($\%_{col}$, $\%_{row}$)	54 (7.5, 55.7)	43 (9.4, 44.3)
Missing, *n*	1	4

continued

TABLE 17 Baseline data by colonisation status at 2 weeks' postnatal age: infant characteristics (*continued*)

Characteristic	Colonisation with *B. breve* BBG-001	
	Yes (*n* = 724)	No (*n* = 462)
Apgar score 5 minutes after birth		
0–3, *n* ($\%_{col}$, $\%_{row}$)	20 (2.8, 55.6)	16 (3.6, 44.4)
4–6, *n* ($\%_{col}$, $\%_{row}$)	91 (12.8, 59.1)	63 (14.1, 40.9)
7–10, *n* ($\%_{col}$, $\%_{row}$)	598 (84.3, 61.8)	369 (82.4, 38.2)
Missing, *n*	15	14
CRIB II[71]		
n	676	443
Mean (SD)	8.3 (3.4)	9.4 (3.5)
Range	1 to 20	2 to 19
Missing	48	19

b.p.m., beats per minute; CRIB, Clinical Risk Index for Babies; IQR, interquartile range.
a Despite complete data for gestational ages and birthweights there are four missing values for birthweight z-scores. This is because four of the babies were below the reference range of age for any given weight of –0.326 to 23 weeks.

TABLE 18 Other early clinical data collected post randomisation by colonisation status at 2 weeks' postnatal age

Characteristic	Colonised with *B. breve* BBG-001 (*n* = 724)	Not colonised with *B. breve* BBG-001 (*n* = 462)
Enteral feeding in the first 14 days[a]		
Number fed within 14 days of birth	723	455
Postnatal age at first feed (days)		
Mean age (SD)	3.0 (1.6)	3.5 (2.3)
Median age (IQR)	3 (2–4)	3 (2–4)
Range	(1–12)	(1–14)
Type of milk received (0–14 days),* n *(%)		
Any maternal breast milk	691 (95.4)	439 (95.0)
Any donor breast milk	157 (21.7)	99 (21.4)
Any formula	290 (40.1)	130 (28.1)
Maternal breast milk only (0–14 days),* n *(%)		
Yes	307 (42.4)	238 (51.5)
No	417 (57.6)	224 (48.5)
Antacid and antibiotic use (0–14 days),[b] **n *(%)***		
Any antacid given	72 (9.9)	50 (10.8)
Antibiotics given in first 5 days	717 (99.0)	457 (98.9)
Antibiotics given between days 6 and 14	459 (63.4)	374 (80.1)
Total days of antibiotics days 0–14, median (IQR)	8 (3–18)	14 (7–30)

IQR, interquartile range.
a Details of feeds were only collected for the first 14 days after birth.
b Details of medications only collected for the first 14 days after birth.

Primary outcomes by colonisation

Despite the three primary outcomes all being less frequent in those babies who were colonised with *B. breve* BBG-001 at 2 weeks, there was no clear evidence of benefit associated with colonisation. The proportion of infants who had an episode of NEC Bell stage 2 or 3 was 6.5% in the colonised group, compared with 12.6% in the non-colonised group (adjusted RR 0.68, 99% CI 0.43 to 1.09); the corresponding figures for late-onset sepsis were 9.3% and 14.3% (adjusted RR 0.88, 99% CI 0.59 to 1.31) and for death were 3.3% and 7.1% (adjusted RR 0.68, 99% CI 0.35 to 1.29) (*Table 19*).

Secondary outcomes by colonisation

Despite trends towards reduced rates of adverse outcomes in those babies who were colonised at 2 weeks, there was no clear evidence of the benefit for any of the secondary outcomes other than the time to full feeds, which was lower in those infants who were colonised (*Table 20* and *Figure 9*).

TABLE 19 Primary outcomes by colonisation status at 2 weeks' postnatal age

	Colonisation with *B. breve* BBG-001		
Primary outcome	Yes ($n = 724$), *n* (%)	No ($n = 462$), *n* (%)	Adjusted[a] RR (99% CI)
Late-onset sepsis[b]	67 (9.3)	66 (14.3)	0.88 (0.59 to 1.31)
NEC[c]	47 (6.5)	58 (12.6)	0.68 (0.43 to 1.09)
Death	24 (3.3)	33 (7.1)	0.68 (0.35 to 1.29)

a Adjusted for sex, gestational age at birth and randomisation within 24 hours of birth. Centre was excluded, as the model did not converge. Allowances for correlations between multiple births are accounted for.
b Late-onset sepsis is defined as bloodstream infection with non-skin commensals after 72 hours' postnatal age and before 46 weeks' postmenstrual age.
c Necrotising enterocolitis Bell stage 2 or 3.

TABLE 20 Secondary outcomes by colonisation status at 2 weeks' postnatal age

Secondary outcomes	Colonised with *B. breve* BBG-001 ($n = 724$), *n* (%)	Not colonised with *B. breve* BBG-001 ($n = 462$), *n* (%)	Adjusted[a] RR (99% CI)
Late-onset sepsis,[b] NEC[c] or death at discharge home	106 (14.6)	114 (24.7)	0.79 (0.60 to 1.06)
Late-onset sepsis-related and microbiological outcomes			
Positive blood culture for skin commensal	134 (18.5)	130 (28.1)	0.78 (0.60 to 1.01)
Any blood culture taken after 72 hours	517 (71.4)	396 (85.7)	Not converged[d]
Number of blood cultures per infant after 72 hours, median (IQR)	2 (0 to 4)	3 (1 to 6)	0 (0 to 0)
Bloodstream infection by organism			
Enterobacteriaceae	23 (3.2)	23 (5.0)	0.88 (0.42 to 1.82)
Enterococcus	12 (1.7)	13 (2.8)	0.81 (0.29 to 2.22)
Staphylococcus	20 (2.8)	14 (3.0)	1.1 (0.45 to 2.64)

continued

TABLE 20 Secondary outcomes by colonisation status at 2 weeks' postnatal age (*continued*)

Secondary outcomes	Colonised with *B. breve* BBG-001 (*n* = 724), *n* (%)	Not colonised with *B. breve* BBG-001 (*n* = 462), *n* (%)	Adjusted[a] RR (99% CI)
Fungi	5 (0.7)	3 (0.7)	1.09 (0.17 to 7.13)
Other non-skin commensals	19 (2.6)	19 (4.1)	0.83 (0.36 to 1.88)
Antibiotic-resistant bloodstream infection			
MRSA	0	2 (0.4)	Too few data
VRE	0	1 (0.2)	Too few data
ESBL-producing Gram-negative bacteria	2 (0.3)	4 (0.9)	0.30 (0.03 to 2.78)
Gentamicin resistant	1 (0.1)	0	Too few data
Isolates of organisms from other normally sterile sites			
Suprapubic urine	0	1 (0.2)	Too few data
Cerebrospinal fluid	5 (0.7)	6 (1.3)	0.67 (0.14 to 3.27)
Pleural cavity	0	0	
Peritoneum	10 (1.4)	9 (2.0)	0.72 (0.22 to 2.33)
Other (joint fluid)	0	1 (0.2)	Too few data
B. breve BBG-001 from any normally sterile site	0	0	Too few data
Total days of antibiotics after 72 hours, median (IQR)	8 (3 to 18)	14 (7 to 30)	5 (4 to 7)
Total days of antifungals after 72 hours, median (IQR) [range]	0 (0 to 0) [0 to 154]	0 (0 to 3) [0 to 58]	0 (0 to 0)
Feeding and growth			
Reached full feeds, *n* (%)	718 (99.2)	447 (96.8)	
Died before reaching full feeds	4 (0.6)	13 (2.8)	
Postnatal age at first full feed (150 ml of milk/kg/day), days			
Median	13	17	1.36 (1.16 to 1.59)[e]
99% CI of median	12 to 14	15 to 18	
IQR	9 to 19	11 to 26	
Change in weight z-score (from baseline to 36 weeks' postmenstrual age)			
n	721	460	
Mean (SD)	–1.33 (0.92)	–1.47 (0.91)	0.08 (–0.05 to 0.21)
Range	–6.76 to 2.69	–6.02 to 1.23	

TABLE 20 Secondary outcomes by colonisation status at 2 weeks' postnatal age (*continued*)

Secondary outcomes	Colonised with *B. breve* BBG-001 (*n* = 724), *n* (%)	Not colonised with *B. breve* BBG-001 (*n* = 462), *n* (%)	Adjusted[a] RR (99% CI)
Other morbidities			
Survivors to 36 weeks' postmenstrual age, *n*	699	428	
Bronchopulmonary dysplasia (any O_2 at 36 weeks' postmenstrual age)	238 (34.1)	192 (44.9)	0.80 (0.67 to 0.95)
Severe bronchopulmonary dysplasia at 36 weeks' postmenstrual age[f]	79 (11.3)	72 (16.8)	0.94 (0.66 to 1.34)
n	645	658	
Hydrocephalus and/or intraparenchymal cysts, *n* (*n*/*N*, %)[g]	38 (5.3)	40 (8.7)	0.71 (0.41 to 1.24)
n	691	441	
Worst stage of retinopathy of prematurity ≥ stage 3 in either eye, *n* (*n*/*N*, %)	23 (3.3)	23 (5.2)	0.65 (0.31 to 1.37)
Length of stay			
n	724	459	
Total length of hospital stay (days), median (IQR)	64 (46 to 91)	75 (53 to 104)	9 (4 to 14)
n	724	461	
Intensive care stay (days), median (IQR)	8 (4 to 29)	18 (5 to 39)	4 (2 to 7)
High-dependency unit stay (days), median (IQR)	16 (5 to 31)	23 (9 to 37)	4 (1 to 7)

IQR, interquartile range.

a RRs adjusted for sex, gestational age at birth and randomisation within 24 hours of birth. Centre was excluded as the model did not converge. Allowances for correlations between multiple births are accounted for.

b Late-onset sepsis is defined as bloodstream infection with non-skin commensals after 72 hours postnatal age and before 46 weeks postmenstrual age.

c Necrotising enterocolitis Bell stage 2 or 3.

d Simple unadjusted model does not converge; Fisher's Exact test has a *p*-value of < 0.0001.

e Hazard ratio.

f Severe bronchopulmonary dysplasia was defined as still receiving mechanical ventilatory support or, if breathing spontaneously, still receiving more than 0.1 l/minute oxygen or 30% or higher supplementary oxygen.[72]

g An additional 21 babies (six in the colonised group and 15 in the non-colonised group) had reports of haemorrhagic parenchymal infarcts but never progressed to have reports of hydrocephalus, porencephaly or periventricular leucomalacia, of these one in the colonised and eight in the non-colonised group died before discharge from hospital. *N* = number reporting, if *N* is not specified, the data are complete with no missing items.

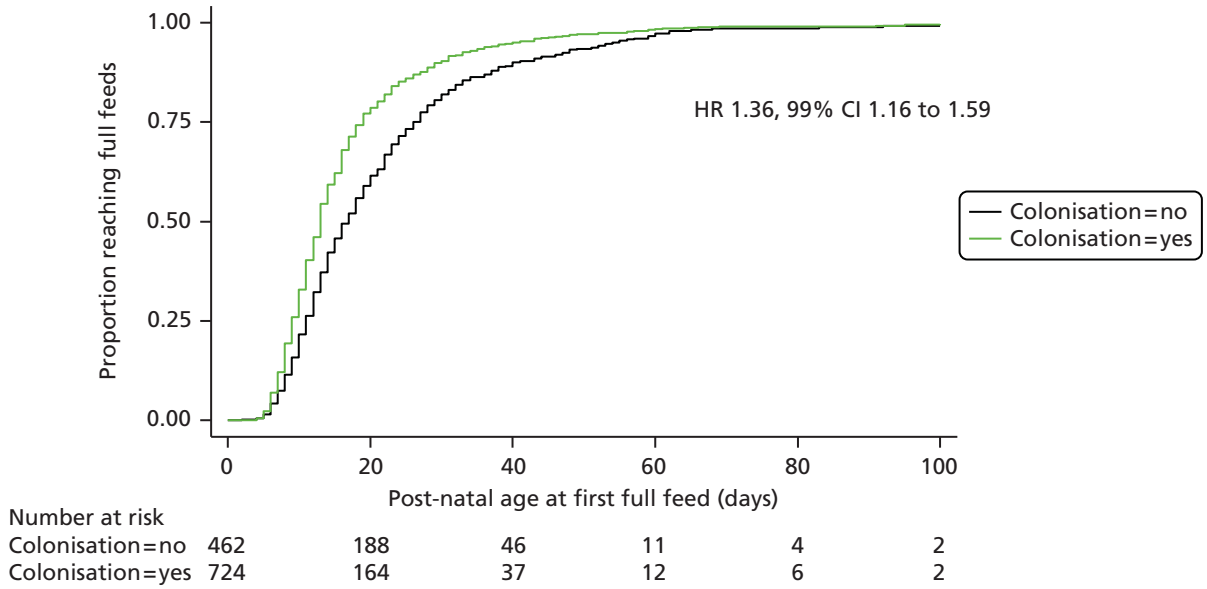


FIGURE 9 Kaplan–Meier plot of time to first feed by colonisation with *B. breve* BBG-001 at 2 weeks' postnatal age. HR, hazard ratio.

Safety

There were no reports of babies not tolerating the interventions. Although detailed data were not collected, our understanding was that those parents who requested discontinuation of the intervention did so because of intercurrent clinical problems, often suspected or proven NEC. We received no requests to 'unblind' the intervention.

There were no reports of positive culture of any bifidobacteria from any normally sterile site.

Analysed by intention to treat, there were no differences in the rates of stool colonisation by antibiotic-resistant bacterial strains either at 2 weeks' postnatal or 36 weeks' postmenstrual age. At 2 weeks, 4.1% of stools from babies colonised with *B. breve* BBG-001 were positive for ESBL-producing Gram-negative bacteria, compared with 1.7% of those not colonised with *B. breve* BBG-001 (*Table 21*); this difference is not statistically significant. At 36 weeks' postmenstrual age, the rate of ESBL-producing Gram-negative bacterial colonisation was 3.8% in the colonised babies and 3.3% in the non-colonised babies.

There were two reports of SAEs (*Table 22*): one baby allocated to the placebo group suffered fatal toxic epidermal necrolysis and one baby allocated to the probiotic group survived a massive pulmonary haemorrhage that was initially, apparently incorrectly, thought to be associated with a transfusion reaction. Neither SAE was considered likely to be related to the interventions. There were no reports of SUSARs.

TABLE 21 Secondary outcomes by colonisation status at 2 weeks' postnatal age: stool microbiology

Secondary outcome	Colonised with *B. breve* BBG-001 ($n = 724$), *n* (%)	Not colonised with *B. breve* BBG-001 ($n = 462$), *n* (%)	Adjusted[a] RR (99% CI)
2 weeks' postnatal age			
MRSA, *n* (%)	3 (0.4)	0	Too few data
VRE, *n* (%)	1 (0.1)	0	Too few data
ESBL-producing Gram-negative bacteria *n* (%)	30 (4.1)	8 (1.7)	1.86 (0.72 to 4.80)
36 weeks' postmenstrual age[b]			
MRSA, *n* (*n*/*N*, %)	1 (0.2)	0	Too few data
VRE, *n* (*n*/*N*, %)	1 (0.2)	3 (0.8)	0.21 (0.01 to 4.09)
ESBL-producing Gram-negative bacteria *n* (*n*/*N*, %)	23 (3.8)	13 (3.3)	1.18 (0.49 to 2.82)
Missing	118	69	

a Adjusted for sex, gestational age at birth, randomisation < 24 hours of age. Centre was excluded as the model did not converge. Allowances for correlations between multiple births are accounted for (except for VRE, which is not adjusted for gestational age).
b Fifteen babies died before 36 weeks' postmenstrual age and 172 babies had no stool sample.
N = number reporting, if *N* is not specified, the data are complete with no missing items.

TABLE 22 Serious adverse events by intention to treat

Event	Trial group	
	Probiotic ($n = 650$)	Placebo ($n = 660$)
Toxic epidermal necrolysis (fatal), *n* (%)	0	1 (0.2)
Pulmonary haemorrhage, *n* (%)	1 (0.2)	0

Chapter 5 Discussion

This is the largest trial to date investigating the potential of a probiotic intervention to prevent NEC, late-onset sepsis and death in preterm babies. The probiotic tested was *B. breve* strain BBG-001. The trial was undertaken and completed because, despite the publication of a number of randomised trials and meta-analyses together with some strong recommendations for routine use of probiotics, there were ongoing concerns about the quality of a number of the trials, lack of evidence as to what product might be useful and, in particular, a lack of confidence that the babies included in the trials were representative of the population in UK neonatal units.

The strengths of this trial are the use of a product with a single bacterial strain, which simplifies the interpretation of results; the monitoring of colonisation, so that the extent of successful colonisation of the active intervention group and cross-colonisation of the placebo group is known; and the size of the trial, as it has adequate statistical power to give clear answers about the prevention of NEC and late-onset sepsis. The aim was to recruit babies representative of the total English newborn infant population by having minimal exclusions, and by recruiting and starting the intervention soon after birth.

This is the first trial of a probiotic in the newborn infant to be performed to ICH-GCP standards.

As with other published trials of probiotics, there was no evidence of short-term harm but, in contrast to the sole other large published trial, the Australasian ProPrems trial,[41] and the conclusions of the most recent Cochrane review of this topic,[35] this intervention in this population of babies showed no evidence of benefit; in particular, there was no evidence of prevention of NEC.

This discussion will address the design and conduct of the trial, and the findings in the context of current understanding of the pathogenesis of NEC and severe late-onset sepsis, and it will consider implications for clinical practice and make recommendations for future research.

Trial design and conduct

Duration of trial

This trial relates to the prevention of major complications of preterm birth; the application for funding was made in 2005 with a view to trial staff coming into post in during 2006 with recruitment over 30 months from May 2007. In the event, staff came into post in September 2009, the set-up period was 3 months longer than expected and it took 36 months to complete recruitment. That a trial of such importance for the advancement of neonatal care should be so delayed is important for those planning and funding large trials.

The initial delays were in large part related to regulatory issues around the interventions and were perhaps inevitable. It became clear between the pilot and main trial that the intervention was to be classed as a medicine; this was, we believe, the first application for a probiotic intervention to be granted clinical trial authorisation and there was initially a lack of clarity about the standards that the product should meet.

The delays during the set-up phase and early recruitment related to the NHS research and development (R&D) approval processes and, to a lesser extent, in some hospitals, the lack of staff with GCP training, and are discussed in greater detail in this chapter. Once through these difficult early phases, the rate of recruitment was as had been predicted (see *Figure 3*).

Choice of product

A major criticism of published trials is the failure of the investigators to choose products based on laboratory evidence and evidence from animal studies and preclinical studies in babies supporting their possible efficacy. A problem has been the urgency felt by clinicians to identify interventions to prevent late-onset sepsis and NEC and the lack of such scientific evidence forcing them to select interventions on pragmatic grounds, testing simply what is available or, slightly preferable, a product used in a previous trial that suggested possible efficacy.[73] Our own choice of product was based on the evidence of nutritional advantage in the trial of Kitajima *et al.*,[43] the availability of a placebo, the potential to monitor stool colonisation and the knowledge that the product had been used extensively in Japan and was regarded as being safe. When it became clear that the intervention would be classified as a medicine and that a clinical trial certificate would be necessary, we were fortunate that the product is manufactured to high specification by the manufacturer and that data supporting its stability were available. This was particularly important when it became apparent that there would be difficulty obtaining a second batch of product in order to complete recruitment and an application had to be made to the MHRA to extend the use beyond the original shelf life. The granting of the extension was contingent on our continuing to monitor the viability of the product in each pack and to confirm the absence of contamination. The additional *B. breve* BBG-001 viability data gained (see *Figure 6*) and the absence of any contaminants strengthens confidence in the quality of this product.

Timing of start of intervention

There were a number of reasons why we were keen to begin the intervention early, the most important being that we were keen to be as inclusive as possible and, anecdotally, were conscious that as the clinical course of preterm babies becomes more complex in the days after birth, with events such as episodes of milk intolerance and suspected sepsis, staff and parents may become more reluctant to recruit babies into trials, resulting in the inadvertent exclusion of babies at greatest risk of complications. In addition, whether or not babies are enterally fed in the days after birth, they begin to acquire flora from birth and subsequently through handling and interventions such as passing feeding tubes. If one of the mechanisms by which probiotics might protect against NEC and late-onset sepsis is by modifying the developing flora and discouraging colonisation with potential pathogens, then it seems likely, although evidence is lacking, that efficacy will be enhanced if given early. Some clinicians still withhold feeds from babies at highest risk of NEC (i.e. those babies with growth restriction and evidence of intrauterine hypoxia); thus, delaying the probiotic until some milk is tolerated will inevitably delay its administration. For example, the protocol for the ProPrems trial[74] involved early recruitment within 72 hours of birth but the intervention was not started until at least 1 ml of milk was being tolerated every 4 hours; consequently, the intervention was not started until a median age of 5 days (interquartile range 4–7 days),[41] whereas in the two published trials[39,42] the intervention was started irrespective of feeding at a median age of 2 days[39] and 1 day.[42]

We were successful in that the median age at randomisation in the PiPS trial was 35 hours (see *Table 7*) with the median age at the first dose of intervention being 44 hours; this was earlier than the median age at first feed, which was 3 days.

Not being able to prepare the interventions in whole milk created a difficulty in that, although our powders were completely indistinguishable, the probiotic powder was turbid when made up with water and we had to resort to using dilute milk to prepare them. We are uncertain whether or not the additional precaution of preparing the intervention in amber-coloured bottles was really necessary; none of our investigators ever suggested that they could distinguish between the active intervention and placebo preparations. Although it is not explicit in any of the published trials, several of which used no placebo (see *Table 1*), it seems likely that in some the reason for giving the probiotic intervention in milk was to achieve masking.

In our case the choice of the elemental formula in which to suspend the intervention, Neocate, was made after discussions with gastroenterology and dietitian colleagues. To have used breast milk, which would have been our first choice, was impracticable because of variable availability and opacity. It seemed inconceivable that 1 ml of one-eighth-strength Neocate could impose such a metabolic or immunological stress on the intestinal epithelium that it could cause a clinical problem. We were concerned, however, that the use of any formula might discourage some parents from agreeing to their baby entering the trial. Detailed data were not collected but we regularly questioned our investigator colleagues on whether or not this was a problem. After reassurance that it was believed to be safe and that it would not interfere with successful establishment of breastfeeding, there seemed to be very few instances in which it was a factor in parents refusing to enrol their baby.

Dosage

The trial published by Kitajima *et al.* in 1997[43] is the only other study to have used this product. In that study, the intervention was prepared in 2 ml of water and given in 'two or three' divided doses. We were not keen either to impose this workload on staff or to retain the product for long periods of time once made up. We increased the volume used to suspend the powder from 2 ml to 3 ml to make it easier for staff administering the products to avoid disturbing the maize starch residue when drawing up the 1-ml dose. We estimated that the minimum bacterial count we were likely to find even with a more dilute suspension would be around 6.7×10^7 CFUs, which remains within the range used in previously published trials.

This is the first trial to have monitored the numbers of bacteria that were being given to babies throughout recruitment. This was particularly important since the product was administered beyond the shelf life that had been specified by the manufacturer. We had undertaken to stop recruitment should the number of viable bacteria fall below the threshold recommended by the manufacturer of 2.2×10^8 CFUs (8.3 $\log_{10}$-CFUs) per sachet, despite the fact that the evidence for that number is not clear. The data we received from the laboratory during recruitment all suggested that the bacterial counts were declining at a predictable rate and remaining well above the threshold. The active intervention given to the final recruits was inevitably analysed after the end of the trial and the count in a single pack had fallen to the threshold. There is, however, no evidence that rates of successful colonisation reduced during the trial, and we have no evidence to suggest that the dose was inadequate.

The intervention was stopped at 36 weeks' postmenstrual age rather than continuing it to discharge, as has been done in some trials, as it was felt that parents would prefer it not to be stopped when the baby went home. When babies went home earlier than 36 weeks staff were encouraged, if there was opportunity, to discontinue the intervention a few days before discharge.

Whether or not it is necessary to continue the intervention this long in order to achieve prolonged colonisation has not been systematically studied and is likely to be dependent on the gestational age of the baby, use of antibiotics and feeding. In one small study,[75] it was noted that *L. paracasei* NFBC 338 could still be detected in the stool 2 weeks after a single dose given on day 4 to low-birthweight babies who received no antibiotic subsequent to the probiotic. In the PiPS trial, stools were obtained at just two time points, 2 weeks' postnatal and 36 weeks' postmenstrual age, so we have no information about coming and going of colonisation in between the two time points; however, as 9.5% of the 571 babies in the probiotic group who were successfully colonised at 2 weeks were no longer colonised at 36 weeks' postmenstrual age, we would suggest that in current clinical practice it is probably necessary to continue the intervention at least until the baby is beyond the peak time of risk of NEC, around 32 weeks' postmenstrual age.

None of the previously published trials gives detailed data about exactly how many doses of the intervention babies received, implying, in the majority, that the intervention was given continuously over the prescribed period. Given the anxiety often experienced by nursing staff about giving milk to babies with feed intolerance or any suggestion of early NEC or sepsis, this seems very unlikely. We left the decision of whether or not doses should be omitted to the local clinicians and suggested definite withholding only in the circumstance of suspected intestinal perforation, although we imagined that if a dose of the probiotic was inadvertently given in that situation it could not do any harm. We did give strong encouragement that after resolution of episodes of NEC, if the intervention had been discontinued, it be recommenced, and we are aware that this was done on multiple occasions. We received no reports whatsoever of problems tolerating the interventions and were pleased that the proportion of recommended doses given was high at 85%.

The use of necrotising enterocolitis and late-onset sepsis as trial end points

We had concerns about the objectivity of both NEC and late-onset sepsis as outcomes in clinical trials. The diagnosis of NEC is made on clinical and radiological features (intestinal intramural gas and/or perforation) supported by haematological markers and, in those who die or come to surgery, on the macroscopic and histological appearance of the bowel. The applicability of Bell staging, which for many years has been the method most frequently used to categorise cases, to the contemporary population of very preterm babies with NEC has been challenged,[76] but the method has not been replaced by anything more reliable. The radiological sign of intramural gas can be very difficult to detect, with clinicians disagreeing about its presence; the final diagnosis in non-fatal, non-surgical cases is to a great extent dependent on the total picture and the experience of the clinicians and it was agreed that, for trial purposes, whether or not a baby had the disease and the staging thereof should primarily rest with the attending clinical staff. In practice, this is complicated by the way in which the clinical service is organised with only a small number of neonatal units providing neonatal surgery, which results in babies with suspected or proven NEC frequently moving between hospitals while the clinical picture is evolving. It was for these reasons that we felt that it was essential to review all cases with any abdominal pathology after data collection had ended. This process generated new queries to PIs, particularly when the final diagnosis provided did not fit the detailed data, for example stage 2 NEC with no diagnostic radiological features, and it resulted in changes to the final diagnosis in 10 cases. As a result we are as confident as we feel it is possible to be about the accuracy of the NEC diagnosis, but without this rather labour-intensive process we believe that a number of cases would have been wrongly categorised.

Many of the published trials studying the effect of probiotic interventions on sepsis include in their definition 'any' positive blood culture. One of the great difficulties in studying rates of neonatal sepsis has been reliably distinguishing between positive cultures of *S. epidermidis* that represent true infections and those that arise from contamination from the skin during blood sampling. It is possible that the great range of reported infection rates and the lack of consistency of effect on sepsis is in part attributable to inclusion of non-infected cases with positive blood cultures. The Australasian ProPrems trial,[41] for which sepsis was the primary outcome, addressed this problem by using strict criteria to define infection with *S. epidermidis* involving duplicate positive cultures and duration of antibiotic use. The rationale underpinning the pragmatic approach taken for the PiPS trial, which for the primary outcome excluded all positive cultures of *S. epidermidis,* is described in appendix 1 of the protocol.

As planned, our microbiological outcome data were collected directly from hospital routine laboratories. Although we still believe that we would have had difficulty collecting reliable data about the antibiotic sensitivities of cultured bacteria from neonatal clinicians, this methodology was very laborious and, although we received a high level of support and co-operation from many hospital microbiologists, the process in some hospitals was complicated because of the subcontracting of pathology services. Although, on another occasion, we would follow the same procedure, we would also ask clinicians, in parallel, to report positive blood cultures, since these are unusual and important events and data are likely to be accurate. Such reports would augment and aid interpretation of the often rather complex data received from laboratories that received numerous samples, often seemingly with inadequate information from clinical staff as to their source and the indication for their collection.

The trial population

The gestational age range of eligible recruits (23^{+0} to 30^{+6} weeks' gestation) was selected in order to capture the babies at highest risk of NEC and late-onset sepsis. The importance of gestational age as a risk factor is apparent within the trial data, with all three primary outcomes falling sharply with increasing gestation age within this target range (see *Figure 7*), but the greater number of births at higher gestational age means that the need for effective prevention remains high. Cochrane reviews of the role of probiotics to prevent NEC prior to that published in 2014[30,32] argued that the evidence of efficacy in preterm babies with a birthweight > 1 kg supported routine use. More recently, with publication of the ProPrems trial,[41] that recommendation has been extended to include the extremely low-birthweight group with birthweight < 1 kg. In a recent review of the published literature, Abrahamsson *et al.*[73] are generally critical of the quality of published trials but nonetheless recommend that the most important need is for future trials to address the extremely low-birthweight group. The ProPrems trial[41] did indeed find evidence of decreased NEC in a population of babies born before 32 weeks' gestational age, but the rate of NEC in the placebo group was low, at 4.4%, and the upper 95% CI of the number needed to treat to prevent a single case of NEC was 333. There did appear to be a greater effect in the subgroup with a birthweight > 1 kg (0.3% probiotic vs. 3.2% placebo, compared with 4.3% probiotic vs. 5.9% placebo in the extremely low-birthweight group), but the numbers are small and the interaction *p*-value was 0.08. We would still argue that the case for routine use in the UK for babies of birthweight > 1 kg is not made and that the population included in the PiPS trial is the one we need to study.

Participating hospitals

The recruiting hospitals in the PiPS trial were all in or close to London, UK. The clinical service is organised into networks, with the care of the majority of babies born before 27 weeks' gestation being in the larger tertiary centres. The recruiting hospitals included both tertiary and secondary centres in order to ensure a spread of gestational ages within the population. During the course of the trial some hospitals further afield expressed enthusiasm to join, but by that time the organisation of the trial was established and we did not have the capacity to increase the number of recruiting centres. The initial decision to confine the trial to the south-east of England was made for two reasons. The first was that in the early planning stages it was expected that the recruitment period for the PiPS trial would overlap with the randomised trial of oxygen targeting, the Benefits of Oxygen Saturation Targeting, Clinical Trial (BOOST),[77] which involved many major neonatal centres but none in London. In the event, the beginning of recruitment to PiPS was delayed and hospitals close to London that had recruited to BOOST joined. The second was that considerable personal support by the team of trial research nurses would clearly be needed to both recruiting and 'step-down' centres in making up the interventions. This would have been more difficult and expensive had they been further afield. The alternative would have been to fund research nurses in participating centres but, with an intervention that continued until 36 weeks' postmenstrual age and a pattern of clinical care that involves babies moving between intensive and lower-dependency units, it was concluded that that was impractical.

Setting the trial up and early recruitment problems

We had planned for a 6-month period from the beginning of the trial until the start of recruitment but required 9 months. The principal reasons for this were delays in gaining NHS R&D approvals and, in a number of hospitals, the lack of anyone on the staff who had received GCP training and could act as PI or take informed consent. Our original plan, because we knew that the babies would move between hospitals during the course of the intervention, was not to begin recruitment at any hospital in a network until all hospitals in that network had the necessary approvals in place. This would unquestionably have been an advantage, but such were the delays at some centres that it was not practicable. When a baby was recruited we asked which hospitals he/she might be transferred to in the hope that we could expedite the gaining of approvals in those places. This, however, was only partially successful, and in the early stages of recruitment a number of babies had to discontinue the intervention prematurely because they were transferred to a non-approved site.

Particularly in smaller hospitals, staff found it difficult to get time off to attend GCP courses when the online course was unavailable. As a consequence, few of the smaller hospitals had more than one person, or possibly two people, who could take consent. This problem was compounded by local NHS trust rules preventing nurses, however experienced and familiar with the trial they were, from taking consent in a trial involving a medicinal intervention.

These problems resulted not only in delay in opening sites (see *Table 3*) but also in very slow early recruitment (see *Figure 3*), so that after 6 months it had to be questioned whether or not the trial could be delivered. The possibility of engaging more recruiting sites was rejected and with a programme of constant hands-on encouragement and training from trial staff, coupled with support from the Medicines for Children Network staff based at Great Ormond Street Hospital in identifying research nurses, recruitment accelerated so that the total duration was only 6 months longer than originally planned.

The development of standardised procedures for the governance of research in the NHS over the past 20 years has greatly increased the quality and accountability of much clinical research but the resultant bureaucracy can at times be obstructive and still needs to be honed further. The extreme that we encountered was the situation of a hospital in which the staff were keen to collaborate and act as a continuing care site but who were prevented from doing so by their trust, which, because of the financial implications, would agree only if it could be a recruiting centre. Any baby recruited into the trial who was transferred to that hospital for step-down care had to discontinue the intervention despite the parents having agreed for their baby to receive the intervention until 36 weeks' postmenstrual age and the willingness of the staff to administer it.

Less dramatic, but more frequent, and equally disruptive to the conduct of the trial, were examples of babies who were transferred to hospitals that could not have been predicted and the trial staff encountering such extremes of obstructive behaviour and seeming lack of insight into the importance of protocol adherence on the part of local R&D staff, who argued that their sole interest was to protect the patients, that approval could not be gained in time for the intervention to be continued smoothly. It was sometimes possible to accelerate things by the chief investigator ringing personally, on occasion to the hospital's director of R&D, but this really should not be necessary and was not always possible or even effective.

Results

Identification of Bifidobacterium breve *BBG-001*

This is the first multicentre trial of a probiotic intervention in the newborn infant systematically to have studied the presence of the administered probiotic strain in stools as a marker of intestinal colonisation. It was beyond the scope of this trial to collect stools more frequently and so the data serve to provide a snapshot at the two selected time points. The 2-week time point was selected as the basis for the secondary analysis of primary outcomes by 'colonisation' because it was thought that in the majority of cases it would be before the onset of NEC and it was speculated that if the intervention was to provide protection it would perhaps have needed to colonise the intestine by around this time. We have no data for the presence of *B. breve* in the stools between these two time points, but it is clearly possible that, even within the group given the probiotic, colonisation may come and go as, of those babies with samples at both time points, 54 (63%) of the 86 babies who were not colonised at 36 weeks' postmenstrual age had been colonised at 2 weeks. The selective medium used for culture was provided by the manufacturer and said to be strain specific; it was validated at species level by MALDI-TOF. It was decided early in the trial, if there was sufficient stool, to augment culture with PCR because of concerns that it was sometimes taking several days for samples to reach the laboratory. The PCR is based on a method reported by the manufacturer and again is described by the manufacturer as specific for the intervention strain. PCR, in the context of this trial, has the advantage not only that it might detect bacteria that have died during any delay in reaching the laboratory but also that it might detect bacteria whose growth is inhibited in culture by antibiotic given to the baby.

The proportion not colonised by culture but found to be colonised by PCR was 38% in the *B. breve* group and 18% in the placebo group. We have not separately evaluated the PCR in comparison with culture and cannot exclude the possibility that the PCR might have identified some strains inaccurately and be giving a high false-positive rate. In selecting strains of commensal bacteria to develop for commercial use, manufacturers will inevitably choose those that colonise readily. It seems unlikely that the high rates of cross-colonisation we found are confined to *B. breve* BBG-001 but, in the absence of such detailed colonisation data using any other probiotic products, we cannot comment further.

Successful colonisation by Bifidobacterium breve *BBG-001 of babies in the probiotic arm of the trial*

Despite the early administration of the probiotic intervention, using both culture and PCR we detected *B. breve* BBG-001 in the stools of only 85% of babies in the probiotic arm of the trial at 2 weeks' postnatal age. We did not have the resource to do PCR at 36 weeks' postmenstrual age; the number of those positive by culture alone had risen from 74% at 2 weeks to 84%.

The technique of PCR appears to be very sensitive and, although we were surprised not to detect higher colonisation at 2 weeks, there are multiple reasons why colonisation may not be complete. Antibiotic use in these babies, at least in the first few days after birth, was almost universal. Furthermore, over 10% received an antacid in the first 2 weeks. We were surprised that this number was so high, and it is likely that this had an impact on the acquisition of bacterial flora but the detail has not been researched. It was encouraging that over 90% of infants received some maternal breast milk, as the bifidogenic properties of breast milk are well recognised; however, the actual volumes received by many babies were likely to have been small. It is interesting that in the univariable analysis of determinants of colonisation (see *Table 15*) babies were disadvantaged by starting feeds later and seemed to be particularly advantaged, in this respect, if receiving a combination of breast and formula milk. One can speculate that this might be attributable to faster advancement of volumes of feeds or, possibly, to the bifidogenic properties of the breast milk being augmented by the addition of oligosaccharides to preterm formula. None of these feeding effects remained significantly associated with colonisation in the multivariable models, for which the overwhelming effects were the negative impact of prolonged antibiotic use and the advantage of higher gestational age.

The determinants of interactions between probiotic bacteria and the human infant gut are comparatively poorly understood.[78] For example, at the present time the extent to which newborn infants at different gestational ages express the specific epitopes required for binding of probiotics is not known. Adhesion to cell surface-associated structures has been shown to be an important determinant of metabolic and immune interactions for both commensal and pathogenic bacteria in animal models of colonisation and disease.[79,80] Motherway *et al.*[81] described type IVb tight adherence pilus expression and associated genetic determinants in *B. breve* UCC2003 using a mouse model of colonisation. The epitopes for pilus adhesion in this mouse model have yet to be identified. Specific human mucus binding pili have been described in the probiotic strain *L. rhamnosus* GG.[82] It is known that skin structures are poorly developed in infants born before 34 weeks' gestation, that the skin develops rapidly in the few weeks after birth but that in the most preterm infants a fully functional stratum corneum may not have developed by 4 weeks' postnatal age.[83] It may be that the lack of benefit associated with enteral supplementation with *B. breve* strain BBG-001 in this study reflects the immaturity of the gastrointestinal tract of the preterm infant in the early weeks of life. Further research may help to elucidate the importance of adhesion and other types of probiotic interaction in determining preterm infant outcomes.

Colonisation by Bifidobacterium breve *BBG-001 of the placebo arm of the trial*

In order to minimise the possibility of cross-contamination, local nursing staff received training from the trial staff emphasising the importance of completing preparation of the intervention for each baby, then cleaning all working surfaces before progressing to the next. In addition, staff were reminded of the importance of maintaining strictly routine infection control procedures in clinical areas. Despite these precautions, *B. breve* BBG-001 was detected by culture in 49% of infants in the placebo group by 36 weeks' postmenstrual age (*Table 13*).

Pooled (probiotic- plus placebo-allocated babies) colonisation data by site were monitored by the trial management team throughout recruitment. We were conscious that cross-colonisation was taking place, as from a number of sites around 70% of stool samples were colonised. We were looking out for any sites in which there was a clear excess of colonised babies, but because of the movement of babies between sites and interpretation of small numbers it was never possible to be confident that any individual unit was a 'high outlier'. Rather than target additional training at specific hospitals, we informed investigators that it was clear from the data that cross-colonisation was occurring and we reminded them of the importance of taking precautions.

The rates we found of colonisation of babies allocated to receive placebo were similar to those reported in the study of Kitajima *et al.*[43] using the same product (44% at 6 weeks after birth) and in our own pilot study (35% at 4 weeks after birth).

Colonisation data have recently been reported for a subset of babies (five in the probiotic group, seven in the placebo group and 31 non-enrolled babies) from a single site involved in the ProPrems trial.[41,84] The intervention included three bacterial strains, *S. thermophilus* TH-4, *B. infantis* BB-02 and *B. lactis* BB-12; stools were analysed by PCR and the presence of at least two of three strains was classified as cross-colonisation. All of the babies allocated to receive probiotic, one of those allocated to receive placebo and two of the other babies were colonised at postnatal ages ranging from 35 to 174 days. At first sight this rate of cross-colonisation seems low compared with our own, but the numbers are too small to be confident about this and it is not clear why the definition of colonisation involved two rather than one bacterial species.

It is most unlikely that cross-colonisation is unique to the PiPS trial.

The combined effects of incomplete colonisation of the active probiotic with only partial colonisation of the placebo group might lead to underestimation of any potential benefit. The intention-to-treat analysis undertaken for subgroups categorised by colonisation status does not suggest, however, that efficacy in this trial is impacted by cross-colonisation (see *Figure 7*). Nor, in the analysis of primary outcomes by colonisation at 2 weeks (see *Table 19*), is there clear evidence of advantage associated with *B. breve* BBG-001 colonisation.

Probiotics in Preterm infantS trial results: outcomes

We found no evidence of benefit for this probiotic intervention in this population of preterm babies for any of the three primary outcomes or for any of the secondary outcomes, which included measures of the severity and time of onset of NEC, other measures of late-onset sepsis and a range of important neonatal morbidities.

The failure to show a reduction in NEC or mortality is at variance with a number of meta-analyses,[27,30–32,35,63] including the most recent Cochrane review, and with the recently published large multicentre ProPrems trial[41] that recruited in Australia and New Zealand and which, while failing to find evidence of benefit for either sepsis or mortality, did show a protective effect for NEC (2.0% active intervention group vs. 4.4% placebo group). Interestingly, the time of onset of NEC Bell stage ≥ 2 at a postmenstrual age of 30 weeks is earlier in the PiPS trial than in the data based on a Canadian population, but is similar to preliminary data for time of onset of serious (surgical and/or fatal) NEC at a median postmenstrual age of 30 weeks (interquartile range 28–32 weeks' postmenstrual age) in a 2-year prospective cohort of 14,294 babies born between 23 and 31 completed weeks of gestation admitted to neonatal units in England in 2012 and 2013.[85]

Exposure to maternal corticosteroid and early maternal breast milk, both protective against NEC, was high in both the PiPS and the ProPrems trials, but the rates of NEC and pathogen-related sepsis are higher in in the placebo group in this trial (NEC 10.0% vs. 4.4% and pathogen-related sepsis 11.7% vs. 8.7%). The baseline data suggest that a higher proportion of the babies in the PiPS trial are small for their gestational age, which might account for some of this difference. Variations in rates of major morbidities in preterm populations are, however, well recognised and often difficult to explain, most likely being related to variation in baseline risk factors and characteristics of the clinical service. One comment that has been made in a number of commentaries on the possible routine use of probiotics is that probiotics might have greater effect in populations with 'higher' rates of NEC, but this is not supported by our findings.

We undertook subgroup analyses (see *Figure 7*), as described in the trial statistical analysis plan (see *Appendix 12*), together with an additional analysis to aid comparison with the ProPrems data,[41] with the participants categorised by gestational age < 28 weeks versus ≥ 28 weeks. These subgroup analyses are important because in the most recent Cochrane review[35] the previous recommendation,[32] which stated that probiotics should be routinely used in those with a birthweight > 1 kg but that more evidence of efficacy was needed at lower birthweight, was revised and extended to suggest that routine use be considered at all birthweights. The ProPrems trial subgroup analyses by birthweight and gestational age suggested evidence of efficacy to reduce both NEC and sepsis in those of birthweight > 1 kg and 28 weeks' gestation, but not in the smaller, less mature, babies. In contrast to the results of the ProPrems trial, we found no evidence of trends towards a decrease of NEC associated with *B. breve* BBG-001 with increasing gestation or birthweight. Inspection of the data suggests a trend towards lower rates of sepsis in those allocated to receive probiotic, but this does not reach statistical significance. The subgroup analyses do show two statistically significant effects: decreased rates of sepsis in the probiotic group (2.8%) compared with the placebo group (7.3%) (adjusted RR 0.39, 95% CI 0.16 to 0.96) for those born at 28 or 29 weeks of gestation and increased mortality in the probiotic group (12 cases, 3.6%) compared with the placebo group (three cases, 0.9%) (adjusted RR 3.93, 95% CI 1.14 to 13.56). However, a large number of tests have been performed, and the numbers are small, and these results should be treated with caution. The interaction tests for the subgroup analyses are all statistically underpowered. A possible confounder in the PiPS trial is the high rate of colonisation by *B. breve* BBG-001 of the placebo group. The intention-to-treat subgroup analysis of the primary outcomes by colonisation status at 2 weeks (see *Figure 7*) does not suggest, however, that efficacy is impacted by cross-colonisation. This conclusion is supported by the secondary analysis of the primary outcomes in those babies from whom a stool sample was received at 2 weeks, not by intention-to-treat but by colonisation status (see *Table 19*), which, while showing trends towards lower rates of sepsis, NEC and death in those colonised, fails to reach a significant difference for any outcome. The same is true for the analyses of secondary outcomes by colonisation status at 2 weeks, except for statistically significant results showing earlier achievement of full feeds and suggesting reduction of bronchopulmonary dysplasia associated with successful colonisation (see *Table 20*).

The conclusion of the logistic regression analysis performed to study determinants of successful colonisation in those infants who were given *B. breve* BBG-001, that increasing gestation is the most important determinant of successful colonisation and use of antibiotics beyond the fifth day after birth the most important association of failure to colonise, may be interpreted to suggest that in this trial population successful colonisation is perhaps simply a marker of babies at lower clinical risk of adverse outcomes and that this explains the trends towards fewer complications when analysing by colonisation.

Much of the reason for the strong recommendations of some authors for routine probiotic use is the finding of the meta-analyses of significant reduction in all-cause mortality.[35] Together, the ProPrems[41] and PiPS trials have recruited over 2400 babies, which is over one-third of all babies recruited into over 20 probiotic trials. That neither ProPrems nor PiPS found any evidence of benefit to reduce mortality in intention-to-treat analyses has to raise serious concerns about the findings of the meta-analyses and consequent recommendations.

The secondary analysis of outcomes by colonisation at 2 weeks was planned because we expected that, because of the previous report of Kitajima *et al.*[43] and our own pilot study, we would find considerable cross-colonisation of the placebo group and that any therapeutic impact of the intervention would be clear only by studying outcomes in the population successfully colonised irrespective of their treatment allocation. That we found trends towards lower rates of the primary outcome and a range of secondary outcomes but that they still failed to reach statistical significance confirms the lack of evidence of efficacy of this particular probiotic intervention to reduce complications of prematurity in this population.

Safety

As in all other trials of probiotic interventions in the newborn infant, we received no reports of problems administering the intervention and no reports of positive cultures of any bifidobacteria.

We encouraged clinicians to ensure that hospital microbiologists knew that this intervention was being given, but are aware from our contacts with microbiologists to collect culture results that the message was not always relayed around departments. The few reports there have been of bifidobacteria sepsis suggest that the infection is usually very mild and easily treated. It is not clear that anaerobic cultures are performed on all specimens or, if it is grown, if laboratory staff are unaware that a baby is receiving bifidobacteria they might be dismissed as contaminants on a routine culture. It is possible that in this and in other trials bifidobacteraemia is missed and under-reported. Nonetheless, even if this is the case, short-term safety appears to be good.

There is interest in the possibility that probiotics might be useful to reduce carriage of antibiotic-resistant pathogens,[86] but, conversely, theoretically, by increasing microbial diversity, they might increase pathogen carriage. Our results were reassuring in this respect. Although, overall, around 6% of babies from whom we received stools were colonised at one or other time point with MRSA, VRE or ESBL, there was no evidence of difference between the groups whether categorised by allocation or colonisation.

Longer-term safety data for probiotic interventions in preterm babies are almost completely lacking. There is a reassuring report of growth and development[87] of babies followed up to the age of 3 years from one of the earlier trials conducted in Taiwan[20] but no trial has been designed with adequate power to look at important long-term health outcomes including atopic disease. The ProPrems trial[41] has a follow-up component that promises to begin to fill this void, but those results are not yet available.

Generalisability of the results

A major objective when designing the PiPS trial was to ensure the representativeness of the trial population. Records of all potentially eligible babies, the precise numbers of parents who were approached and reasons for refusal for their baby to join the trial were not required by the trial protocol. The view of the investigators was that such data are often inaccurate and we were conscious that, for this trial, the overwhelming reason for eligible babies not being recruited would be the lack of a staff member available who was familiar with the trial and who also had GCP training. We aimed to ensure that those who were approached were representative of the total preterm population by minimising exclusions and both recruiting for and beginning the intervention early, making it more difficult to exclude babies for spurious clinical reasons, which, purely anecdotally, we suspect often happens.

The PiPS trial population is strikingly multiracial, with the mothers of 56% of the babies being white, 20% being Afro-Caribbean and 12% with origins in the Indian subcontinent. Race is not recognised as a risk factor for the outcomes of this trial, but the racial mix might be related to the observation that the average birthweight was slightly low for gestational age and it is well recognised that intrauterine growth restriction is a risk factor for both NEC and late-onset sepsis. The gestational age-based mortality of the babies in the trial (see *Figure 7*) is very similar to that reported nationally for neonatal unit admissions in 2009 in England[88] (data from 110 neonatal units with details to discharge or death for 34,635 babies born before 33 weeks' gestational age): 60% mortality rate at 23 weeks' gestational age, 38% at 24 weeks' gestational age, 27% at 25 weeks' gestational age, 16% at 26 weeks' gestational age,

11% at 27 weeks' gestational age, 6% at 28 weeks' gestational age, 4% at 29 weeks' gestational age and 2% at 30 weeks' gestational age. NEC lacks a standard agreed case definition and the incidence is not available from UK routine national data; the recording of episodes of infection is incomplete on the national database. The rates that we found in the trial for NEC and late-onset sepsis in the placebo group (10.0% and 11.7%, respectively) were within the ranges that we had specified in the power calculation that were based on routine data collected for neonatal admissions in and around London in the years preceding the trial and similar to rates quoted in large observational studies.[59,60] On balance, we believe that the trial population is certainly representative of the population in south-east England and we are not aware of any good reasons why these results should not be extended to neonatal populations in the rest of the country.

Why does the probiotic intervention in the Probiotics in Preterm infantS trial show no evidence of benefit? Probiotics in Preterm infantS in the context of other trials in preterm babies

In the context of the variable effects on sepsis and lack of effect in multiple meta-analyses we attempted to clarify the role of *B. breve* BBG-001 to reduce late-onset sepsis by, for our primary outcome, considering only septicaemias caused by definite pathogens. That we found no benefit is disappointing but not unexpected. It was perhaps more surprising that the ProPrems trial[41] showed no evidence of improved mortality associated with the three-strain probiotic intervention. In the context of that trial, which we would argue is the first large well-designed preterm probiotic trial to be published, it is possibly less surprising that we similarly found no evidence of reduced mortality associated with *B. breve* BBG-001 administration in the PiPS trial. However, despite our criticisms and those of others of the quality of many of the published trials,[34,73] we were surprised that we have been able to find no evidence of benefit to reduce NEC in a clinical environment with an incidence of definite NEC (Bell stage ≥ 2) of around 10%. At first sight it might be thought that we have missed an effect because of loss of statistical power through incomplete colonisation of the active intervention group and cross-colonisation of the placebo group, but our intention-to-treat subgroup analyses by colonisation and analyses of outcomes by colonisation do not support this view. Furthermore, we were forewarned of this as a potential problem and went to great lengths to provide training to minimise the effect; it seems most unlikely that either cross-infection rates in the hospitals involved in the PiPS trial are very different from elsewhere or that this has not also been a characteristic of other trials of probiotics.

The results of the PiPS trial might be negative in the sense of identifying a preventative therapy but they are most important in the story of probiotic trials and the search for a means to prevent NEC and late-onset sepsis.

There are many reasons why this intervention might have failed.

In the context of the wide range of products used in previously published trials[34,63,73] it is too simplistic to simply dismiss this result as being because we chose the wrong product. The evidence we have, as discussed earlier, from laboratory, animal and limited human data all suggests that different bacterial strains have different protective roles at the intestinal mucosal surface; this is biologically plausible and explains why we are dependent on such an enormous range of commensals for good health. The increased availability of molecular techniques to study the microbiome has led, over the period since the PiPS trial was designed in 2004–6, to an explosion in knowledge of the microbiome, not only in health but in a range of disease states including NEC and late-onset sepsis in the preterm baby.[15–17,89,90] That explosion of knowledge is not confined to the bacteria that inhabit the intestine but also includes other compartments of the body, most notably for the neonatologist, maternal breast milk[91] and the complexity of interactions between the microbiome and the immature intestinal immune system that underpin the pathophysiology of NEC.[4,92,93] Although we remain convinced that the key to unlocking the challenge of preventing these neonatal catastrophes lies in this area of biology, it seems naive from the current vantage point to think that this might be achieved by giving a product with a single or even two or three bacterial strains.

Combining trials of different probiotics in meta-analyses

The very strong and often emotional pressure that has been put on clinicians by a number of authors to provide probiotics routinely for preterm babies has been based on no single well-designed large trial but on a series of meta-analyses, the most recent of which was the Cochrane review[35] published in 2014, which included 23 trials and over 5500 babies. Other authors have repeatedly challenged not only the rigour of a number of the trials but also the validity of combining trials using such disparate interventions in meta-analyses. We would argue that the results of the PiPS trial further strengthen the argument that combining trials using different probiotic interventions in meta-analyses should be resisted.

Conclusions

Implications for health care

Necrotising enterocolitis and late-onset sepsis remain among the most important causes of death and life-long morbidity in surviving preterm babies. Rates of preterm birth in the UK are among the highest in the developed world[94] and are rising;[95] therefore, preterm birth represents a major public health issue and the challenge to find interventions to prevent NEC and late-onset sepsis is urgent. We are aware that there is currently a rise in the use of probiotics on UK neonatal units given to prevent NEC. The data from this trial do not support this practice. The short-term safety profile for probiotic interventions for the individual baby is highly favourable, but there are almost no published outcome data beyond the initial hospitalisation, and the use of probiotics on a neonatal unit will impact not only on the developing microbiome of the babies for whom they are prescribed but also, through cross-colonisation, on that of other babies within that unit.

A number of commentaries have suggested that it is perhaps unethical not to inform parents about the evidence of probiotics to prevent NEC[29] and that, when given the evidence, parents would be likely to elect that their baby should be given them.[96] This, however, obviously depends on the interpretation of the data by the responsible clinician, the manner in which he or she presents it to the parents and the availability of a product with adequate quality control. The data from this trial provide no evidence that routine supplementation with *B. breve* BBG-001 would affect the risk of late-onset sepsis, NEC or death in this population.

Recommendations for research

Regulatory considerations and neonatal trials

Although there has been progress in standardising and centralising procedures to ease the conduct of trials, it remains necessary to gain local hospital approvals for those involving inpatients. This is a particular problem for trials recruiting preterm babies, whose clinical care is organised in such a way that they inevitably move between hospitals, sometimes along unpredictable pathways, and it was a major threat to the success of this trial.

This problem may be eased if GCP training was provided for all medical neonatal trainees and consultants, and for interested nurses, who could additionally be able to act as the PI for trials of IMPs, although some hospitals have local rules preventing this. Furthermore, if a baby is transferred after any intervention is complete and the only requirement is to collect outcome data, the procedures for approved members of the trial team to access case notes could be further simplified.

Standardisation of definitions of outcomes

The diagnosis of both NEC and late-onset sepsis has an element of subjectivity, and neither has an internationally agreed case definition either for epidemiological or for drug regulatory purposes. This complicates the interpretation of individual published neonatal trials and undermines the validity of combining the trials in meta-analyses. The PiPS trial team took a pragmatic approach to this problem, using a definition of late-onset sepsis for the primary outcome that is different from other trials and

undertaking an independent review of the categorisation of NEC. The difficulties are not underestimated; nonetheless, the lack of agreed simple practical definitions is a major obstacle to progress and, in conjunction with regulators, needs urgently to be addressed.

The choice of products for probiotic trials

Research into the developmental biology and pathophysiology underlying neonatal NEC and late-onset sepsis progresses and ultimately should point the way to the identification of evidence-based interventions that can be tested in clinical trials to prevent NEC and late-onset sepsis, but that stage has not yet been reached.

The choice of probiotic product for this trial seemed, when made, to have a sound clinical basis but, in retrospect, given progress in understanding the pathogenesis of NEC and late-onset sepsis and the complex biology of the microbiome over the past decade, was perhaps naive.

The conventional route of undertaking large expensive Phase 3 trials only after evidence has been gained from animal and smaller trials in humans supporting efficacy and safety may not be appropriate for probiotic interventions. A bacterium having probiotic properties might behave differently in a different species and its action might vary with the developmental stage. At the very least, however, it would probably be advisable in the future before embarking on large neonatal probiotic trials to complete more formal and bigger 'pilot' studies using clinical end points; however, this is fraught with difficulties and risks being misleading. Such studies might be designed to involve increased longitudinal sampling to give an opportunity to study colonisation and in that way throw increased light on issues of dosage particularly in relation to feeding and antibiotic use.

What is undoubtedly important is that product quality is monitored and colonisation rates are documented throughout and not just during the set-up phase of clinical trials.

Randomised controlled trials to evaluate probiotics

We do not consider that efficacy of *B. breve* BBG-001 to prevent NEC and late-onset sepsis was missed in the PiPS trial because of the complication of cross-colonisation; nonetheless, cross-colonisation is probably a feature of all probiotic trials and complicates their interpretation. The problem could be overcome by adopting a cluster design although even that precaution, in the context of unpredictable patterns of transfer within the UK neonatal service, might not totally overcome the problem and in addition has major organisational implications.

The well-designed RCT is the supreme assessment of efficacy since it provides a clean experiment with all confounders taken into account. To suggest that it might not be the optimal way to evaluate a probiotic and to suggest that the conclusions from a trial of a probiotic might be less likely to translate into clinical practice than those of a trial of a stable chemical entity may seem heretical, but these suggestions are worthy of consideration. We can only speculate, but from what we have found it seems inevitable that if a probiotic is introduced into routine practice it will be incorporated into the bacteriological environment of the neonatal unit. We are unaware of any longitudinal studies of the effects of prolonged routine use, if any, on the microbiome in terms of its diversity and frequency of antibiotic-resistant strains in a population of babies, but this is critically important. It is indeed possible not only that an intervention that seemingly shows efficacy in a trial might lose that efficacy over time but equally that an intervention that shows no evidence of benefit might, over time, promote bacterial diversity and be associated with reduced rates of NEC and late-onset sepsis. The same may be true whether or not the intervention is safe.

Studying probiotic efficacy and safety outside a randomised controlled trials

It is inconceivable that all of the outstanding questions about choice and combinations of probiotic and other bioactive interventions can be answered through RCTs.

The pathogenesis of NEC and of neonatal late-onset sepsis is complex, involving the interplay of the acquisition of a microbiome that is different from that of the full-term healthy baby, with functionally immature intestinal and immune systems, and, superimposed upon that, effects of enteral nutrition and administered medications such as antibiotics and antacids.

There is a strong association between the prolonged use of antibiotics and NEC and it is known that the provision of maternal breast milk affords some protection. Furthermore, there is accumulating evidence that the use of evidence-based care bundles may reduce neonatal adverse outcomes including late-onset sepsis and NEC[97,98] and it has also been shown in the UK that, through enthusiastically supported quality improvement programmes, the use of maternal breast milk may be increased.[99] Breast milk itself is a safe means to provide not only commensal bacteria and bifidogenic oligosaccharides, but also a range of other factors including lactoferrin and growth factors.

In England, all neonatal units routinely collect a common clinical data set, an anonymised subset of which is held in the Neonatal Data Analysis Unit at Imperial College London.[100] Those data items, the completeness and accuracy of which are steadily increasing, constitute a kite-marked research data set (ISB1595) that includes both interventions and outcomes. These have been successfully linked to Hospital Episode Statistics and have the potential to link to other child health systems, opening up possibilities for obtaining longer-term outcome data. This rich data source has already been the basis of a number of important publications and has huge potential for investigating the effects and the interactions of interventions such as probiotics, prebiotics and other bioactive products that are likely to need testing and whose effects might change over time. Ensuring the completeness and accuracy and the quality of the analysis of these 'routine' data may yield more knowledge about the true impact of introducing probiotics, or any other intervention, than the best-designed RCT.

Summary of research recommendations

1. Further streamline training of research competent clinical and NHS R&D staff to ensure that recruitment is optimised and adherence to trial protocols is not unnecessarily jeopardised by transfer between hospitals.
2. Standardise objective case definitions for NEC and late-onset sepsis suitable for epidemiological, trial and regulatory purposes.
3. Consider undertaking pilot trials with clinical outcomes and embedded longitudinal studies of colonisation before embarking on large Phase 3 trials of probiotics.
4. During probiotic trials, monitor the quality of the intervention and intestinal 'colonisation'.
5. If designing RCTs of probiotics, consider cluster design to limit the effects of cross-colonisation.
6. If introducing probiotic (or other bioactive) interventions into routine use, set up long-term longitudinal monitoring of the microbiome and of clinical outcomes in the neonatal population whether or not prescribed probiotic.
7. Ensure the completeness and accuracy of routine NHS neonatal data so that it reliably forms the basis for studies of the multiple influences on acquisition of NEC and late-onset sepsis.

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Contributions of authors

All authors have been involved in the production of this report and have approved the final manuscript.

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Pollyanna Hardy (Senior Trials Statistician, NPEU). Oversight of production of statistical analysis plan, analysis and interpretation of results.

Paul Heal (PiPS Trial Co-ordinator, NPEU). Day-to-day running of trial including regulatory aspects, training of trial staff and data collection and collation.

Edmund Juszczak (Director, NPEU Clinical Trials Unit). Trial design and oversight, statistical advice and interpretation of results.

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Michael R Millar (Consultant in Infection, Barts Health NHS Trust; PiPS trial investigator). Original hypothesis, trial design and oversight, lead for collection of clinical microbiology and analysis and interpretation of results.

Publications

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Millar MR, Seale J, Turton J, Wilks M, Costeloe K, Woodford N, *et al.* ESBL-producing Enterobacteriaceae in 24 neonatal units and associated networks in the south of England: no clustering of ESBL-producing *Escherichia coli* in units or networks. *J Antimicrob Chemother* 2016;**71**:1174–7.

Data sharing statement

All available data relating to this trial can be obtained from the corresponding author via The Senior Trials Manager, The National Perinatal Epidemiological Unit, Nuffield Department of Population Health, University of Oxford, Old Road Campus, Headington, Oxford OX3 7LF, UK.

References

1. Stoll BJ, Gordon T, Korones SB, Shankaran S, Tyson JE, Bauer CR, *et al.* Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1996;**129**:63–71. http://dx.doi.org/10.1016/S0022-3476(96)70191-9
2. Lin PW, Stoll BJ. Necrotising enterocolitis. *Lancet* 2006;**368**:1271–83. http://dx.doi.org/10.1016/S0140-6736(06)69525-1
3. Hintz SR, Kendrick DE, Stoll BJ, Vohr BR, Fanaroff AA, Donovan EF, *et al.* Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. *Pediatrics* 2005;**115**:696. http://dx.doi.org/10.1542/peds.2004-0569
4. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med* 2011;**364**:255–64. http://dx.doi.org/10.1056/NEJMra1005408
5. Walsh MC, Kliegman RM. Necrotising enterocolitis: treatment based on staging criteria. *Pediat Clin North Am* 1986;**33**:179–201.
6. Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr* 1999;**28**:19–25. http://dx.doi.org/10.1097/00005176-199901000-00007
7. Gewolb IH, Schwalbe RS, Taciak VL, Harrison TS, Panigrahi P. Stool microflora in extremely low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1999;**80**:F167–73. http://dx.doi.org/10.1136/fn.80.3.F167
8. Sakata H, Yoshioka H, Fujita K. Development of the intestinal flora in very low birth weight infants compared to normal full-term newborns. *Eur J Pediatr* 1985;**144**:186–90. http://dx.doi.org/10.1007/BF00451911
9. Schwiertz A, Gruhl B, Lobnitz M, Michel P, Radke M, Blaut M. Development of the intestinal bacterial composition in hospitalized preterm infants in comparison with breast-fed, full-term infants. *Pediatr Res* 2003;**54**:393–9. http://dx.doi.org/10.1203/01.PDR.0000078274.74607.7A
10. Duffy LC. Interactions mediating bacterial translocation in the immature intestine. *J Nutr* 2000;**130**:432S–6S.
11. Walker WA. Role of nutrients and bacterial colonization in the development of intestinal host defense. *J Pediatr Gastroenterol Nutr* 2000;**30**(Suppl. 2):2–7. http://dx.doi.org/10.1097/00005176-200000002-00002
12. Kohler H, McCormick BA, Walker WA. Bacterial–enterocyte crosstalk: cellular mechanisms in health and disease. *J Pediatr Gastroenterol Nutr* 2003;**36**:175–85. http://dx.doi.org/10.1097/00005176-200302000-00005
13. Martin CR, Walker WA. Probiotics: role in pathophysiology and prevention in necrotizing enterocolitis. *Semin Perinatol* 2008;**32**:127–37. http://dx.doi.org/10.1053/j.semperi.2008.01.006
14. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *FASEB J* 2001;**15**:1398–403. http://dx.doi.org/10.1096/fj.00-0833hyp
15. Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, *et al.* Fecal microbiota in premature infants prior to necrotizing enterocolitis. *PLOS ONE* 2011;**6**:e20647. http://dx.doi.org/10.1371/journal.pone.0020647

16. Stewart CJ, Marrs ECL, Magorrian S, Nelson A, Lanyon C, Perry JD, *et al.* The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection. *Acta Paediatr* 2012;**101**:1121–7. http://dx.doi.org/10.1111/j.1651-2227.2012.02801.x

17. Mai V, Torrazza RM, Ukhanova M, Wang X, Sun Y, Li N. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. *PLOS ONE* 2013;**8**:e52876. http://dx.doi.org/10.1371/journal.pone.0052876

18. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization. *Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*. FAO Food and Nutrition Paper 85. 2001. URL: www.fao.org/3/a-a0512e.pdf (accessed 25 August 2016).

19. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. *Biol Neonate* 2002;**82**:103–8. http://dx.doi.org/10.1159/000063096

20. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, *et al.* Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005;**115**:1–4.

21. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, *et al.* Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr* 2005;**147**:192–6. http://dx.doi.org/10.1016/j.jpeds.2005.03.054

22. Samanta M, Sarkar M, Ghosh P, Ghosh JK, Sinha MK, Chatterjee S. Prophylactic probiotics for prevention of necrotizing enterocolitis in very low birth weight newborns. *J Trop Pediatr* 2009;**55**:128–31. http://dx.doi.org/10.1093/tropej/fmn091

23. Lin H-C, Hsu C-H, Chen H-L, Chung M-Y, Hsu J-F, Lien R-I, *et al.* Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 2008;**122**:693–700. http://dx.doi.org/10.1542/peds.2007-3007

24. Neu J, Caicedo R. Probiotics: protecting the intestinal ecosystem? *J Pediatr* 2005;**147**:143–6. http://dx.doi.org/10.1016/j.jpeds.2005.05.033

25. Kliegman RM, Willoughby RE. Prevention of necrotizing enterocolitis with probiotics. *Pediatrics* 2005;**115**:171–2.

26. Bell EF. Preventing necrotizing enterocolitis: what works and how safe? *Pediatrics* 2005;**115**:173–4.

27. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. *Lancet* 2007;**369**:1614–20. http://dx.doi.org/10.1016/S0140-6736(07)60748-X

28. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, *et al.* Enteral feeding of premature neonates with *Saccharomyces boulardii*. *Early Hum Dev* 2003;**74**:89–96. http://dx.doi.org/10.1016/S0378-3782(03)00090-2

29. Tarnow-Mordi WO, Wilkinson D, Trivedi A, Brok J. Probiotics reduce all-cause mortality and necrotizing enterocolitis: it is time to change practice. *Pediatrics* 2010;**125**:1068–70. http://dx.doi.org/10.1542/peds.2009-2151

30. AlFaleh KM, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Sys Rev* 2008;**1**:CD005496. http://dx.doi.org/10.1002/14651858.cd005496.pub2

31. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics* 2010;**125**:921–30. http://dx.doi.org/10.1542/peds.2009-1301

32. AlFaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Sys Rev* 2011;**3**:CD005496. http://dx.doi.org/10.1002/14651858.cd005496.pub3

33. Soll RF. Probiotics: are we ready for routine use? *Pediatrics* 2010;**125**:1071–2. http://dx.doi.org/10.1542/peds.2010-0643

34. Mihatsch WA, Braegger CP, Decsic T, Kolacek S, Lanzinger H, Mayer B, *et al.* Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. *Clin Nutr* 2012;**31**:6e15.

35. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Sys Rev* 2014;**4**:CD005496. http://dx.doi.org/10.1002/ebch.1976

36. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of *Bifidobacterium lactis* on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. *Neonatology* 2010;**98**:156–63. http://dx.doi.org/10.1159/000280291

37. Sari FN, Dizdar EA, Oguz S, Erdeve O, Uras N, Dilmen U. Oral probiotics: *Lactobacillus sporogenes* for prevention of necrotizing enterocolitis in very low-birth weight infants: a randomized, controlled trial. *Eur J Clin Nutr* 2011;**65**:434–9. http://dx.doi.org/10.1038/ejcn.2010.278

38. Braga TD, Pontes da Silva GA, Cabral de Lira PI, de Carvalho Lima M. Efficacy of *Bifidobacterium breve* and *Lactobacillus casei* oral supplementation on necrotizing enterocolitis in very-low-birth-weight preterm infants: a double-blind, randomized, controlled trial. *Am J Clin Nutr* 2011;**93**:81–6. http://dx.doi.org/10.3945/ajcn.2010.29799

39. Rojas MA, Lozano JM, Rojas MX, Rodriguez VA, Rondon MA, Jaime A, *et al.* Prophylactic probiotics to prevent death and nosocomial infection in preterm infants. *Pediatrics* 2012;**130**:e1113–20. http://dx.doi.org/10.1542/peds.2011-3584

40. Fernández-Carrocera LA, Solis-Herrera A, Cabanillas-Ayón M, Gallardo-Sarmiento RB, García-Pérez CS, Montaño-Rodríguez R. Double-blind, randomised clinical assay to evaluate the efficacy of probiotics in preterm newborns weighing less than 1500 g in the prevention of necrotising enterocolitis. *Arch Dis Child Fetal Neonatal Ed* 2013;**98**:F5–9. http://dx.doi.org/10.1136/archdischild-2011-300435

41. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, *et al.* Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. *Pediatrics* 2013;**32**:1055–62. http://dx.doi.org/10.1542/peds.2013-1339

42. Oncel MY, Sari FN, Arayici S, Guzoglu N, Erdeve O, Uras N, *et al. Lactobacillus reuteri* for the prevention of necrotising enterocolitis in very low birthweight infants: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* 2014;**99**:F110–15. http://dx.doi.org/10.1136/archdischild-2013-304745

43. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial *Arch Dis Child* 1997;**76**:F101–7. http://dx.doi.org/10.1136/fn.76.2.F101

44. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. *Int J Infect Dis* 1999;**3**:197–202. http://dx.doi.org/10.1016/S1201-9712(99)90024-3

45. Luoto R, Matomäki J, Isolauri E, Lehtonen L. Incidence of necrotizing enterocolitis in very-low-birth-weight infants related to the use of *Lactobacillus* GG. *Acta Paediatr* 2010;**99**:1135–8. http://dx.doi.org/10.1111/j.1651-2227.2010.01795.x

46. Hunter C, Dimaguila MAVT, Gal P, Wimmer JE Jr, Ransom JL, Carlos RQ, *et al.* Effect of routine probiotic, *Lactobacillus reuteri* DSM 17938, use on rates of necrotizing enterocolitis in neonates with birthweight < 1000 grams: a sequential analysis. *BMC Pediatrics* 2012;**12**:142. http://dx.doi.org/10.1186/1471-2431-12-142

47. Bonsante F, Iacobelli S, Gouyon J-B. Routine probiotic use in very preterm infants: retrospective comparison of two cohorts. *Am J Perinatol* 2013;**30**:41–6.

48. Janvier A, Malo J, Barrington KJ. Cohort study of probiotics in a North American neonatal intensive care unit. *J Pediatr* 2014;**164**:980–5. http://dx.doi.org/10.1016/j.jpeds.2013.11.025

49. Manzoni P, Lista G, Gallo E, Marangione P, Priolo C, Fontana P, *et al.* Routine *Lactobacillus rhamnosus* GG administration in VLBW infants: a retrospective, 6-year cohort study. *Early Hum Dev* 2011;**87S**:S35–8. http://dx.doi.org/10.1016/j.earlhumdev.2011.01.036

50. Kunz AN, Noel JM, Fairchok MP. Two cases of *Lactobacillus* bacteremia during probiotic treatment of short gut syndrome. *J Pediatr Gastroenterol Nutr* 2004;**38**:457–8. http://dx.doi.org/10.1097/00005176-200404000-00017

51. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. *Lactobacillus* sepsis associated with probiotic therapy. *Pediatrics* 2005;**115**:178–81.

52. Ohishi A, Takahashi S, Ito Y, Ohishi Y, Tsukamoto K, Nanba Y, *et al. Bifidobacterium* septicaemia associated with postoperative probiotic therapy in a neonate with omphalocele. *J Pediatr* 2010;**156**:679–81. http://dx.doi.org/10.1016/j.jpeds.2009.11.041

53. Jenke A, Ruf E-M, Hoppe T, Heldmann M, Stefan W. *Bifidobacterium* septicaemia in an extremely low-birthweight infant under probiotic therapy. *Arch Dis Child Fetal Neonatal Ed* 2012;**97**:F217–18. http://dx.doi.org/10.1136/archdischild-2011-300838

54. Murguia-Peniche T, Mihatsch WA, Zegarra J, Supapannachart S, Ding Z-Y, Neu J. Intestinal mucosal defense system, part 2. Probiotics and prebiotics. *Pediatrics* 2013;**162**:S64–71. http://dx.doi.org/10.1016/j.jpeds.2012.11.055

55. Li Y, Shimizu T, Hosaka A, Kaneko N. Ohtsuka Y, Yamashiro Y. Effects of *Bifidobacterium breve* supplementation on intestinal flora of low birth weight infants. *Pediatr Int* 2004;**46**:509–15. http://dx.doi.org/10.1111/j.1442-200x.2004.01953.x

56. Chrzanowska-Liszewska D, Seliga-Siwecka J, Kornacka MK. The effect of *Lactobacillus rhamnosus* GG supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial. *Early Hum Dev* 2012;**88**:57–60. http://dx.doi.org/10.1016/j.earlhumdev.2011.07.002

57. Besselink MG, van Santvoort HC, Buskens E, Boermeester MA, van Goor H, Timmerman HM, *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. *Lancet* 2008;**371**:651–9. http://dx.doi.org/10.1016/S0140-6736(08)60207-X

58. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, *et al.* Late onset sepsis in very low birth weight neonates: the experience of the NICHD neonatal research network. *Pediatrics* 2002;**110**:285–91. http://dx.doi.org/10.1542/peds.110.2.285

59. Llanos AR, Moss ME, Pinzòna MC, Dye T, Sinkin RA, Kendiga JW. Epidemiology of neonatal necrotising enterocolitis: a population based study. *Paediatr Perinatal Epidemiol* 2002;**16**:342–9. http://dx.doi.org/10.1046/j.1365-3016.2002.00445.x

60. Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. *Semin Perinatol* 2008;**32**:70–82. http://dx.doi.org/10.1053/j.semperi.2008.01.004

61. Underwood MA, Kalanetra KM, Bokulich NA, Lewis ZT, Mirmiran M, Tancredi DJ, *et al.* A comparison of two probiotic strains of bifidobacteria in premature infants. *J Pediatrics* 2013;**163**:1585–91. http://dx.doi.org/10.1016/j.jpeds.2013.07.017

62. Ishizeki S, Sugita M, Takata M, Yaeshima T. Effect of administration of bifidobacteria on intestinal microbiota in low-birth-weight infants and transition of administered bifidobacteria: a comparison between one-species and three-species administration. *Anaerobe* 2013;**23**:38–44. http://dx.doi.org/10.1016/j.anaerobe.2013.08.002

63. Wang Q, Dong J, Zhu Y. Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials. *J Pediatr Surg* 2012;**47**:241–8. http://dx.doi.org/10.1016/j.jpedsurg.2011.09.064

64. Costeloe KL, Jesudass R, Al Nakib L, Whiley A, Wilks M, Millar MR. The early administration of *Bifidobacterium breve* strain BBG to very low birthweight infants: a pilot study. *Pediatr Res* 2004;**55**:2802.

65. Neu J. Neonatal necrotizing enterocolitis: an update. *Acta Paediatr* 2005;**94**(Suppl. 449):100–5. http://dx.doi.org/10.1080/08035320510043637

66. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK. Incidence and timing of presentation of necrotizing enterocolitis in preterm infants. *Pediatrics* 2012;**129**:e298–304.

67. European Parliament. *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community Code Relating to Medicinal Products for Human Use*. Article 1. Brussels: European Parliament; 2001.

68. Costeloe K, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, *et al. PiPS: Trial of Probiotic Administered Early to Prevent Infection and Necrotising Enterocolitis*. Protocol. 2012. URL: www.nets.nihr.ac.uk/__data/assets/pdf_file/0012/51213/PRO-05-501-04.pdf (accessed 25 August 2016).

69. British Society for Antimicrobial Chemotherapy (BSAC). *BSAC Methods for Antimicrobial Susceptibility Testing*. URL: http://bsac.org.uk/wp-content/uploads/2012/02/Version-12-Apr-2013_final1.pdf (accessed 25 August 2016).

70. Fujimoto J, Tanigawa K, Kudo Y, Makino H, Watanabe K. Identification and quantification of viable *Bifidobacterium breve* strain Yakult in human faeces by using strain-specific primers and propidium monoazide. *J Appl Microbiol* 2011;**110**:209–17. http://dx.doi.org/10.1111/j.1365-2672.2010.04873.x

71. Parry G, Tucker J, Tarnow-Mordi W. CRIB II: an update of the clinical risk for babies score. *Lancet* 2003;**361**:1789–91. http://dx.doi.org/10.1016/S0140-6736(03)13397-1

72. Jobe AH, Bancalari E. NICHD/NHLBI/ORD workshop summary: bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;**163**:1723–9. http://dx.doi.org/10.1164/ajrccm.163.7.2011060

73. Abrahamsson TR, Rautava S, Moore AM, Neu J, Sherman PM. The time for a confirmative necrotizing enterocolitis probiotics prevention trial in the extremely low birth weight infant in North America is now! *J Pediatr* 2014;**165**:389–94. http://dx.doi.org/10.1016/j.jpeds.2014.05.012

74. Garland SM, Tobin JM, Pirotta M, Tabrizi SN, Opie G, Donath S, *et al.* The ProPrems trial: investigating the effects of probiotics on late onset sepsis in very preterm infants. *BMC Infect Dis* 2011;**11**:210–19. http://dx.doi.org/10.1186/1471-2334-11-210

75. McGee R, O'Connor PM, Russell D, Dempsey EM, Ryan AC, Ross PR, *et al.* Prolonged faecal excretion following a single dose of probiotic in low birth weight infants. *Acta Paediatr* 2010;**99**:1587–8. http://dx.doi.org/10.1111/j.1651-2227.2010.01878.x

76. Gordon PV, Swanson JR, Attridge JT, Clark R. Emerging trends in acquired neonatal intestinal disease: is it time to abandon Bell's criteria? *J Perinatol* 2007;**27**:661–71. http://dx.doi.org/10.1038/sj.jp.7211782

77. BOOST-II UK (Benefits of Oxygen Saturation Targeting) Trial. *Protocol and Handbook*. Oxford: National Perinatal Epidemiology Unit; 2011. URL: https://www.npeu.ox.ac.uk/downloads/files/boost/BOOSTII-Protocol-Version-3-Nov-2011.pdf (accessed 25 August 2016).

78. Ventura M, Turroni F, Motherway MO, MacSharry J, van Sinderen D. Host–microbe interactions that facilitate gut colonization by commensal bifidobacteria. *Trends Microbiol* 2012;**20**:467–76. http://dx.doi.org/10.1016/j.tim.2012.07.002

79. Kline KA, Falker S, Dahlberg S, Normark S, Henriques-Normark B. Bacterial adhesins in host–microbe interactions. *Cell Host Microbe* 2009;**5**:580–92. http://dx.doi.org/10.1016/j.chom.2009.05.011

80. Krachler AM, Orth K. Targeting the bacteria–host interface: strategies in anti-adhesion therapy. *Virulence* 2013;**4**:284–94. http://dx.doi.org/10.4161/viru.24606

81. Motherway MO, Zomer A, Leahy SC, Reunanen J, Bottacini F, Claesson MJ, *et al.* Functional genome analysis of *Bifidobacterium breve* UCC2003 reveals type IVb tight adherence (Tad) pili as an essential and conserved host-colonization factor. *Proc Natl Acad Sci USA* 2011;**108**:11217–22. http://dx.doi.org/10.1073/pnas.1105380108

82. Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, *et al.* Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human mucus binding protein. *Proc Natl Acad Sci USA* 2009;**106**:17193–8. http://dx.doi.org/10.1073/pnas.0908876106

83. Kalia YN, Nonato LB, Lund CH, Guy RH. Development of skin barrier function in premature infants. *J Invest Dermatol* 1998;**111**:320–6. http://dx.doi.org/10.1046/j.1523-1747.1998.00289.x

84. Hickey L, Garland SM, Jacobs SE, O'Donnell CPF, Tabrizi SN. Cross-colonization of infants with probiotic organisms in a neonatal unit. *J Hosp Infect* 2014;**88**:226e–9. http://dx.doi.org/10.1016/j.jhin.2014.09.006

85. Battersby C, Santhakumaran S, Modi N, Costeloe K. Incidence and outcomes of severe necrotising enterocolitis in infants less than 32 weeks gestation: a prospective population study. *Arch Dis Child* 2014;**99**(Suppl. 2):A38. http://dx.doi.org/10.1136/archdischild-2014-307384.112

86. Gibson MK, Pesesky MW, Dantas G. The yin and yang of bacterial resilience in the human gut microbiota. *J Mol Biol* 2014;**426**:3866–76. http://dx.doi.org/10.1016/j.jmb.2014.05.029

87. Chou I-C, Kuo H-T, Chang J-S, Wu S-F, Chiu H-Y, Su B-H, *et al.* Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. *J Pediatrics* 2010;**156**:393–6. http://dx.doi.org/10.1016/j.jpeds.2009.09.051

88. Neonatal Data Analysis Unit. *Neonatal Specialist Care in England: Report on Mortality in 2009*. London: Neonatal Data Analysis Unit; 2010. URL: www1.imperial.ac.uk/resources/E8F81103-48A7-44D4-A914-424EFF22470F/ndaureportmortality2009.pdf (accessed 1 March 2016).

89. Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH, *et al.* Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed* 2012;**97**:F456–62. http://dx.doi.org/10.1136/fetalneonatal-2011-301373

90. Carlisle EM, Morowitz MJ. The intestinal microbiome and necrotizing enterocolitis. *Curr Opin Pediatr* 2013;**25**:382–7. http://dx.doi.org/10.1097/MOP.0b013e3283600e91

91. Jeurink PV, van Bergenhenegouwen J, Jiménez E, Knippels LMJ, Fernández L, Garssen J, *et al.* Human milk: a source of more life than we imagine. *Benef Microbes* 2013;**4**:17–30. http://dx.doi.org/10.3920/BM2012.0040

92. Cilieborg MS, Boye M, Sangild PT. Bacterial colonization and gut development in preterm neonates. *Early Human Dev* 2012;**88**:S41–9. http://dx.doi.org/10.1016/j.earlhumdev.2011.12.027

93. Neal MD, Sodhi CP, Dyer M, Afrazi B, Richardson WM, Beer-Stolz D, *et al.* A critical role for TLR4 induction of autophagy in the regulation of enterocyte migration and the pathogenesis of necrotizing enterocolitis. *J Immunol* 2013;**190**:3541–51. http://dx.doi.org/10.4049/jimmunol.1202264

94. MacDorman MF, Mathews TJ, Mohangoo AD, Zeitlin J. International comparisons of infant mortality and related factors: United States and Europe, 2010. *National Vital Statistics Reports* 2014;**63**:1–7.

95. Zeitlin J, Mohangoo AD, Delnord M, Cuttini M. The second European Perinatal Health Report: documenting changes over 6 years in the health of mothers and babies in Europe. *Epidemiol Community Health* 2013;**67**:983–5. http://dx.doi.org/10.1136/jech-2013-203291

96. Deshpande G, Rao SC, Keil AD, Patole SK. Evidence-based guidelines for use of probiotics in preterm neonates. *BMC Medicine* 2011;**9**:92–104. http://dx.doi.org/10.1186/1741-7015-9-92

97. Montgomery-Taylor S, Emery F, Anthony M. Eighteen months of 'Matching Michigan' at a UK neonatal intensive care unit. *Pediatr Infect Dis J* 2014;**32**:565–7. http://dx.doi.org/10.1097/INF.0b013e3182868389

98. Lee SK, Shah PS, Singhal N, Aziz K, Synnes A, McMillan D, *et al.* Association of a quality improvement program with neonatal outcomes in extremely preterm infants: a prospective cohort study *CMAJ* 2014;**186**:E485–94. http://dx.doi.org/10.1503/cmaj.140399

99. Battersby C, Santhakumaran S, Upton M, Ladbone L, Birch J, Modi N. The impact of a regional care bundle on maternal breast milk use in preterm infants: outcomes of the East of England quality improvement programme. *Arch Dis Child Fetal Neonatal Ed* 2014;**99**:F395–40. http://dx.doi.org/10.1136/archdischild-2013-305475

100. Imperial College. *Neonatal Data Analysis Unit*. URL: www1.imperial.ac.uk/departmentofmedicine/divisions/infectiousdiseases/paediatrics/neonatalmedicine/ndau/ (accessed 1 March 2016).

Appendix 1 Probiotics in Preterm infantS trial sites

Recruiting (24):

Barnet Hospital
Basildon Hospital
Croydon University Hospital
Homerton University Hospital
John Radcliffe Hospital, Oxford
King's College Hospital, London
Luton and Dunstable Hospital
Medway Maritime Hospital
Newham University Hospital
North Middlesex University Hospital
Queen's Hospital, Romford
Royal Sussex County Hospital, Brighton
Southend Hospital
St George's Hospital, London
St Peter's Hospital, Chertsey
St Thomas' Hospital, London
The Royal London Hospital
Tunbridge Wells Hospital at Pembury
University College Hospital
University Hospital Lewisham
Watford General Hospital
Whipps Cross Hospital
Whittington Hospital
William Harvey Hospital, Ashford, Kent

Continuing Care (33):

Addenbrooke's Hospital, Cambridge
Bedford Hospital
Chase Farm Hospital
City Hospital Birmingham
Colchester General Hospital
Darent Valley Hospital, Dartford
Frimley Park Hospital
Great Ormond Street Hospital for Children
Hinchingbrooke Hospital
Horton General Hospital
King George Hospital, Ilford
Lister Hospital
Norfolk and Norwich Hospital
Northampton General Hospital
Northwick Park Hospital, Harrow
Peterborough Hospital

Continuing Care (continued):

Princess Royal Hospital, Haywards Heath
Princess Royal University Hospital
Queen Elizabeth Hospital, Woolwich
Queen Elizabeth Queen Mother Hospital
Queen Mary's Hospital, Sidcup
Royal Berkshire Hospital
Royal Free
Royal Surrey County Hospital
Sandwell General Hospital
Stoke Mandeville Hospital
The Great Western Hospital, Swindon
The Princess Alexandra Hospital
West Middlesex University Hospital
West Suffolk Hospital
Wexham Park Hospital, Slough
Worthing Hospital
Wythenshawe Hospital, Manchester

Appendix 2 Guidance sheet 3: preparation and administration of the trial intervention

PiPS
Probiotic in Preterm babies Study

Guidance Sheet 3: Preparation and administration of the Trial Intervention

- The active intervention in this trial is a live probiotic bacterium, *B. breve* BBG. The product is supplied as a freeze dried powder with corn starch. The placebo is freeze dried corn starch alone. A package **must only be used** by the baby for whom it is allocated.

Cross contamination

- In previous trials of probiotics, colonisation of participants with the bacterial strains under investigation has been found in some of the participants of the placebo arm. Cross colonisation of babies undermines the quality of the trial data.

- Cross colonisation may occur during preparation of the intervention or between babies in clinical areas of the ward and it is important that you are vigilant to the possibility of cross contamination. In the PiPS trial we will monitor rates of colonisation of babies. The procedures for the intervention preparation are designed to minimise this possibility.

- Cross contamination is most likely to occur when two or more babies require intervention preparation. It is important to avoid contamination between different babies' preparations of the intervention. Each time you prepare the intervention **you must ensure that**:
  - you **do not work between preparations** i.e. steps 6 - 12 should be completed sequentially for a single baby before another baby's preparation is started
  - you **wash your hands and clean the working surface before and after** each individual preparation (i.e. steps 1 and 13)
  - you **do not work between administrations** i.e. steps 15 - 18 should be completed sequentially for a single baby before another baby's administration
  - **after administration** you discard the bijou bottle and syringe then **wash your hands** before the next baby's administration
  - if you spill the intervention powder or solution that it is **thoroughly cleaned up**
  - when finished you discard the unused 1/8 strength Neocate and wash or sterilise the measuring jug and rack ready for use the next day.

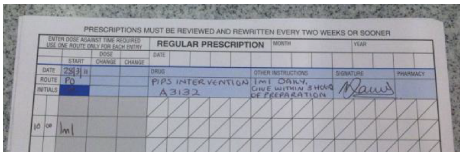
Preparation and administration

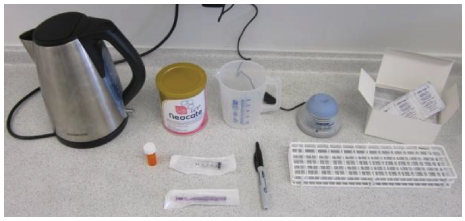
If after the intervention has settled it is inconvenient to administer it, it can be left for 2.5 hours and it will remain stable but you must ensure it is given within the **3 hour time limit** by checking the time recorded on the bijou bottle. After this time it should be discarded and a new preparation made. **Each wasted or spoiled** preparation or sachet (due to damage, spillage, expiration or contamination) should be recorded in the **Intervention Wastage Log**.

The box below lists the items needed for the trial intervention preparation (obtained from the PiPS Consumables Box) and overleaf describes the process step-by-step.

For the preparation of the trial intervention you will need:

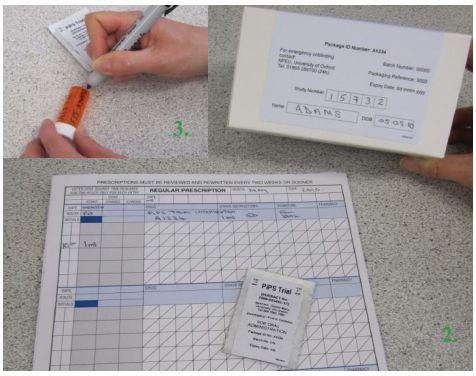
- The baby's drug chart with the prescription for PiPS trial intervention
- The package of PiPS trial intervention allocated to the baby
- Neocate, at least 240mls of cooled boiled water and a clean jug
- An amber 7ml bijou bottle with screw top
- A fine permanent marker pen
- A 5ml syringe
- A 1ml enteral syringe (not provided)
- A 'vortexing' machine to facilitate mixing (this is not essential)
- A rack in which to stand the bijou bottle

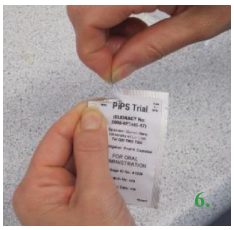


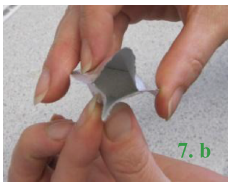


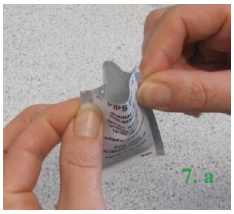
Guidance Sheet 3: Preparation and administration of the Trial Intervention

1. Wash your hands and wipe down the working surface with anti-bacterial wipes.
2. Take one foil sachet of intervention from the package checking:
   - that the number on the sachet, on the package and on the prescription are the same
   - that the name and DOB on the prescription and on the outside of the package are the same
3. Mark the outside of the bijou bottle with the **pack ID number**, the **baby's name** and the **time of preparation** using the marker pen.









4. Prepare 1/8 strength Neocate by adding one scoop of Neocate powder to 240mls of cooled boiled water into the clean jug and mix.
5. Using the 5ml syringe, draw up 3ml of 1/8 strength Neocate and inject it into the bijou bottle.
6. Tear across the top of the foil sachet at the point indicated.
7. Push the edges of the sachet together (**a**) and pinch the label to form a 'spout' (**b**).
8. Carefully pour all of the powder in the sachet into the bijou bottle and screw on the lid firmly.

This is most likely to be associated with some spillage and loss of powder. A minor loss is not important.

9. Discard foil sachet and wash your hands.

10. Agitate the bottle using the vortex mixer for 10 seconds to disperse the powder (**a**). If no vortex is available shake for 30 seconds. Check that the powder is dispersed (**b**); if not repeat as necessary.
11. Stand the bottle in the rack for 30 minutes to allow the corn starch to settle.
12. Wash your hands; clean the working surface and the vortex machine with anti-bacterial wipes.

When preparing intervention for more than one baby you can perform steps 1-5 using the same syringe and Neocate solution before opening any foil sachets.

13. After 30 minutes check the solution has settled out being careful not to disturb the corn starch sediment.
14. Carefully withdraw 1ml of supernatant into the syringe taking care not to disturb the sediment.
15. The volume of supernatant may vary, if after 0.8 ml you are anxious that you might disturb the residue stop and that day give just the 0.8ml.
16. Take the syringe with the sealed bijou bottle to the cotside. Check the **name**, **pack ID number** and **time of preparation** on the bottle with the prescription and the baby's ID tags.
17. Administer the supernatant in the syringe to the baby either via the feeding tube or, if the baby no longer has a tube, directly into the mouth.
18. Record the administration on the baby's **drug chart**.

Appendix 3 Parent information leaflet version 5.1

Introduction

We understand and appreciate that this is a very difficult time for you and that it may not seem a good moment to be talking about research. However we think it is important that you know about a study that this hospital is taking part in for babies born prematurely.

Short Title: PiPS: Probiotic in Preterm babies Study

Formal Title: The probiotic Bifidobacterium breve strain BBG-01 administered early to preterm infants to prevent infection, necrotising enterocolitis and death

Summary

This is a brief description of a research study designed to test whether giving probiotics to premature babies helps to protect them against serious illnesses. You have been given this leaflet because your baby has been, or may be, born 10 or more weeks early and we want to give you the opportunity to think about whether you would like your baby to take part in this study.

Children and adults have many bacteria in their gut that do not cause disease and are important for health. It is possible that if we give similar bacteria (probiotics) by mouth to premature babies soon after birth that other bacteria that can cause illness may be prevented from becoming established in the gut.

The number of babies that have been given probiotics is small; early results of studies are encouraging and probiotics appear to be safe, however we cannot be confident about this until they have been more widely used.

This hospital is one of about 20 helping in a study funded by the NHS through the Health Technology Assessment programme which will involve 1300 very premature babies and give a clear answer about whether probiotics are helpful or not.

The rest of this leaflet explains the study in more detail and describes what being in the study would mean for you and your baby. If, after reading this leaflet and discussing the study with the doctors and nurses in the neonatal unit, you decide to take part we will ask you to sign a consent form. Your baby will then enter the study and will receive either probiotic or an inactive substance that looks the same; this inactive substance is called a placebo. Probiotic or placebo will be continued once a day until shortly before you go home. Neither you nor the staff on the unit will know whether your baby is actually receiving probiotic.

The study does not involve any additional blood tests and should not cause your baby any pain or discomfort. We will collect information about your baby's progress in hospital but we do not currently have any plan to see you again after you have gone home.

Whether or not you decide to let your baby take part is entirely up to you. If you decide not to take part this will not affect the high standard of care your baby receives.

What problem are we trying to help?

Babies born as early as yours are at increased risk of infections and of other complications of prematurity; one of the most important of these is the illness necrotising enterocolitis that affects the gut. Usually these infections can be successfully treated with antibiotics but such illnesses often prolong a baby's stay in hospital and may increase the likelihood of life-long health problems. Occasionally they are so serious that the baby may need surgery or may even die.

There are a number of different ways in which the body protects us against infection; one of the most important is through the millions of bacteria that live in our gut. These are not germs that cause disease but are helpful bacteria that are essential for good health. Babies who are born at the expected time rapidly gain these bacteria from their mother and other members of the family. Babies born early have to be separated from their family and have few of these helpful bacteria; instead they are likely to have many other bacteria in their gut. Usually these other bacteria do not cause problems but they may cause infections that can be difficult to treat and be involved in the development of serious complications such as necrotising enterocolitis. They may also make it more difficult to digest milk which is very important for your baby's long term health.

It is possible that giving probiotics soon after birth will make the bacteria living in the gut of premature babies more like those of full-term babies and decrease the risk of them getting serious infections and necrotising enterocolitis.

What are probiotics and how much do we know about their use in newborn premature babies?

Probiotics are live micro-organisms, usually bacteria, that are taken by mouth and then multiply and live in the gut. There are lots of different probiotic bacteria, many of them have names beginning with Lactobacillus and Bifidobacterium and are contained in live yoghurt and a range of freely available health products.

There have been a number of studies giving probiotics to premature babies. The results suggest that giving probiotic might help babies to digest milk and to grow better. It may also reduce the number of episodes of necrotising enterocolitis and increase survival; the effect upon episodes of bloodstream infection is unclear. However the studies have all been small and none so far has been in the UK. In order to be clear whether or not probiotics are helpful and to be confident of their safety they need to be studied more widely.

Some studies have used just one type of probiotic and others have used mixtures. This study will use a single probiotic called Bifidobacterium breve strain BBG-01 (BBG). For the rest of this leaflet when we talk about probiotic this is the one we mean. The same probiotic has been given routinely to many thousands of newborn babies in Japan.

All of the earlier studies have mixed the probiotic in the baby's milk feeds; this has meant that babies who the doctors decide should not be fed have not been included in the studies. We think it is probably important, if probiotics are to be helpful, that they are given early, before other bacteria that may cause disease become established in the gut. In this study we plan to start probiotics early whether or not milk feeds have been started.

What is the purpose of the study?

The purpose of this study is to find out whether giving BBG to babies born 10 or more weeks early, reduces episodes of blood stream infection and necrotising enterocolitis. We will also study whether there is increased survival and whether babies are likely to leave hospital sooner if they receive probiotic.

Why has my baby been chosen?

All newborn babies are at some risk of infection and of necrotising enterocolitis but this risk is much greater in very premature babies, we are therefore inviting parents of babies born 10 or more weeks early to take part in this study.

This hospital is one of about 20 in England involved with this study. We are aiming to include 1300 babies; we need this number to be confident of finding out whether probiotics are helpful or not.

Does my baby have to take part?

You do not have to agree to your baby taking part in this study. If you decide not to take part it will not affect in any way the quality of care you and your baby receive. Similarly if you decide that you would like your baby to take part and then change your mind your baby can be taken out of the study at any time without you having to give a reason.

What will happen to my baby if I agree to take part?

Because we are studying the effect of giving probiotic early we are asking you to make your initial decision about whether your baby should take part within 48 hours of birth. We realise that this may put you under increased stress and apologise for this; we would not do this if we didn't believe it was important. We will discuss the study with you again during the course of your baby's stay in hospital to make sure that you understand what is happening and that you continue to agree to your baby taking part. There will always be someone available with whom you can discuss the study, sometimes this will be by phone.

If you agree that you would like your baby to take part in this study, your baby will be put into one of two groups; one group will receive probiotic and the other will receive a dummy product that looks the same, this dummy product is called a placebo. Your baby will have a 50/50 chance of being put into either of these groups. The allocation of your baby to a group will rely on chance (rather like tossing a coin). Neither you nor the staff caring for your baby will know which group your baby is in. This is the only way we can be sure that we test probiotic fairly. The first dose of probiotic or placebo will be given as soon as is practicable for the ward staff after you have signed the consent form; this may not be until the following morning.

The probiotic and the placebo are supplied to us as granules which we mix with fluid, we then put a few drops down the baby's feeding tube. We will do this once each day until your baby reaches 36 weeks of gestation (36 + 0 days). Because it is important that nobody knows which product your baby is receiving we mix both probiotic and placebo with a very dilute preparation of a special infant formula called Neocate so that they still look the same. Neocate is an infant formula that is very easy to digest

and is made especially for babies with gut problems; it is not made from cow's milk. For this study Neocate is being used at 1/8 of full strength. This does not provide any significant nutrition for your baby and is so dilute that it cannot pose any risk to the gut even in those babies that the doctors decide should not be fed. This will in no way reduce your chances later of successfully breast feeding your baby.

If your baby is unwell the doctor in charge locally will decide whether or not doses are missed out.

If your baby is discharged home earlier than 36 weeks the probiotic or placebo will be stopped a few days before. If your baby is transferred to a different hospital before 36 weeks we will aim to continue to give the product; if the new hospital is not already involved in the study we will provide training to the staff to enable this to happen.

If your baby sucks well and is able to have the feeding tube removed earlier than 36 weeks the probiotic or placebo will be given directly into the mouth with a syringe once a day before a feed.

Two weeks after birth and again at 36 weeks (if your baby is still in hospital) we will collect a sample of your baby's stool. These samples will be sent to the microbiology laboratory at Barts and the London Hospital, London E1 where they will be tested to check whether or not your baby has been successfully colonised with probiotic and what other bacteria have colonised the gut.

If you agree the remaining stool sample will then be deep frozen and stored for later testing in a related study for which we have not yet secured funding. The additional tests are designed to help us understand the effects of probiotics.

No extra blood tests or injections are necessary and all other aspects of your baby's care will be entirely at the discretion of the local doctors and nurses.

Unless there is a specific medical reason why not, it is hoped that mothers of babies in the study will provide breast milk for their babies since human breast milk promotes the growth of BBG.

What are the possible side effects of the treatment?

In general probiotics are thought to be a very safe treatment. There have been occasional reports of probiotics themselves causing infection; this is extremely unusual and particularly so with the probiotic being used for this study. In the very unlikely event that this should happen the infection would be treated with an antibiotic like penicillin. All babies in the study will be monitored very closely throughout the study by the staff on the Neonatal Intensive Care Unit.

What information will be collected about me and my baby?

We will need to collect standard clinical information about your pregnancy, the condition of your baby at birth and progress throughout the hospital stay. This information will be collected from the baby's written and electronic case record. The study will not involve you in any interviews or questionnaires. In order to get accurate

results from all samples taken by the medical staff to check for infection, we will contact the hospital microbiology laboratory directly since the detail needed for the study is not always available in the case notes.

After your baby has completed the study, records maintained by the NHS Information Centre and NHS Central Register maybe used to help us contact you in future and to provide information about your baby's health status.

What are the possible disadvantages of taking part?

We believe that this intervention is safe and that there are no disadvantages for you in taking part in this study whichever group your baby is in.

We will need to collect information about you and your baby.

What if new information becomes available?

There is currently a lot of interest in the use of probiotics for premature babies and other studies in other countries using slightly different probiotics are underway. We will be monitoring any results emerging from these studies closely and will inform you if any important new information becomes available during the course of the study that might make you change your mind about your baby's involvement.

What if something goes wrong?

The chance of anything going wrong as a result of taking part in this study is very small. However we are required to tell you the following:

If your baby is harmed and this is due to someone's negligence, then you may have grounds for legal action for compensation against Queen Mary, University of London in respect of any harm arising out of the participation in the Clinical Trial or the NHS in respect of any harm which has resulted from the clinical procedure being undertaken.

Will my taking part in this study be kept confidential?

Your GP will be told that you took part in the study.

Your details and the information collected for the study will be kept securely and will only be seen by the study organisers and people from the regulatory authorities whose job is to ensure that studies such as this are carried out safely. They may also need to look at your baby's notes to check that the information collected for the study is correct. Information about you or your baby will not be used for any purpose other than to answer these research questions.

Although we currently have no plans to collect any further information about your baby after discharge from hospital we will retain your contact details in case anything emerges from this or any other study of probiotics that makes it important that we contact you again. The NHS has a central register (based at the General Register Office) that would be able to tell us if you have left the NHS and through which we would be able to locate you.

What will happen to the results of the research?

At the end of the study the results will be analysed and published in an international journal. We will send you a copy of the final results of the study. A copy of the full journal article can be requested from the National Perinatal Epidemiology Unit. You and your baby will not be identified in any report or publication arising from the study.

Who is organising and funding the research?

The study is being run jointly by Barts and the London School of Medicine at Queen Mary, University of London and by the National Perinatal Epidemiology Unit, University of Oxford.

The study is funded by the NHS through the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS records or uses NHS premises or facilities must be approved by a NHS Research Ethics Committee before it goes ahead. Approval cannot guarantee that you will not come to any harm if you take part. However approval does mean that the Committee is satisfied that your rights will be respected, that the risks have been reduced to a minimum and that balanced against the possible benefits it is reasonable for babies born as early as yours to take part. The committee has also checked that we are giving you sufficient information to make an informed decision about taking part.

Thank you for reading this leaflet. The doctor or nurse who gave it to you will be pleased to discuss it with you and to provide further information if that would be helpful. Alternatively the contact details of the study's Principal Investigator in your NHS hospital and the Study Co-ordinator are provided below.

What if I have any concerns?

If at any stage you have any concern or query about this study or the way it has been carried out, you should contact the Principal Investigator (the name and contact details are below), or you may contact the hospital complaints department.

Information is also available on the study website at: www.npeu.ox.ac.uk/pips

If you would like to contact an independent organisation to discuss the inclusion of babies in research studies without reference to this particular study we suggest that you contact the premature baby charity Bliss. Their address is:

Bliss, 9 Holyrood Street, London SE1 2EL
Freephone Family Support Helpline: 0500 618 040
Website: www.bliss.org.uk

Name and contact details of local contact:

Doctors contact details here

Nurses contact details here

Name and contact details of Study Co-ordinator:

Paul Heal (PiPS Trial Co-ordinator)

National Perinatal Epidemiology Unit, Clinical Trials Unit
University of Oxford
XXXX

Tel: XXXX

Appendix 4 Consent form version 3.1

Please complete in black ballpoint pen

PiPS: Probiotic in Preterm babies Study

The probiotic *Bifidobacterium breve* strain BBG–01 administered early to preterm infants to prevent infection, necrotising enterocolitis and death

Professor Kate Costeloe

1. I confirm that I have read and understand the Parent Information Leaflet (version 5.1 dated 14Feb2011) for the above study, and have had the opportunity to ask questions. ☐
2. I understand that the participation of my baby in this study is voluntary and that I am free to withdraw my baby from the study at any time, without giving any reason, without the medical care or legal rights of my baby being affected. ☐
3. I understand that 2 samples of my baby's stools will be retained in the laboratory, for this and future studies, to do tests to help understand the effects of probiotics. ☐
4. I understand that sections of any of the medical notes of my baby may be looked at by responsible individuals from the study organisers or from regulatory authorities where it is relevant to my baby taking part in research. I give permission for these individuals to have access to the records of my baby. ☐
5. I understand that information held by the NHS and records maintained by The NHS Information Centre and the NHS Central Register may be used to help contact me and provide information about my baby's health status. ☐
6. I agree that personal identifying information will be collected, stored and used by the co-ordinating centre to enable follow-up to be undertaken later should it be necessary. This is on the understanding that any information will be treated confidentially. ☐
7. I understand that you will inform my GP that my baby is participating in this study. ☐
8. I agree to my child taking part in the above study. ☐

☐☐/☐☐/☐☐ ☐☐/☐☐/☐☐

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 Consent Form_Version 3.1 dated 28Jan2011

Appendix 5 Leaflet for professionals summarising consent process

PiPS
Probiotic in Preterm babies Study

Obtaining Informed Consent for the PiPS Clinical Trial

This leaflet is for health care professionals who are involved in seeking consent from parent's whose baby is eligible to take part in PiPS.

PiPS_EudraCT No: 2006-003445-17_REC REF: 09/H0604/30 Obtaining Informed Consent_Version 1.0 13Jan10

What is informed consent?

Informed consent is the process by which the parent, after discussing the study with a health care professional, voluntarily confirms their willingness for their baby to participate in the PiPS clinical trial.

Who is eligible to seek informed consent?

Consent can be sought and obtained by any health care professional who has received PiPS and GCP training, and who is registered to do so on the site delegation log. Please confirm with your site Principal Investigator whether you are able to obtain consent.

Who is eligible to give consent?

- Agreement to participate should ideally be sought from both parents of an eligible infant.
- Mothers automatically have parental responsibility for their children and can be the sole signatory on the consent form.
- Fathers may only act as sole signatory on the consent form if married to the mother when the child is born or if named on the birth certificate (**n.b.** the latter is unlikely to be relevant in this trial since it is rare to register the baby this early).
- Unmarried fathers do not automatically have parental responsibility for their child, but a court order or a 'parental responsibility agreement' can give it to them.

When and where should consent be taken?

Although babies can be randomised up to 48 hours after birth we are keen that they are recruited as soon as is feasible. Parents cannot formally give consent before the baby is born but whenever possible staff should try to begin the process of talking to parents about the trial before the onset of labour; the objective is to give them as much time as possible to consider their decision. If this has not been possible then they should be approached as soon as seems reasonable after birth.

If possible consent should be sought in a quiet area of the Unit away from the noise of the monitors and alarms. The person taking consent should use language which is easy to understand and which is free from jargon.

After telling the parent(s) about the trial and giving them an opportunity to ask their immediate questions a Parent Information Leaflet should be left with them to reinforce and expand upon what has been said. The Parent Information Leaflet should list the name and contact details of the local Principal Investigator and the designated local PiPS nurse as well as the contact details for the PiPS Trial Co-ordinator and the preterm baby charity Bliss. Bliss has agreed to be available as a source of independent advice and support for parents; the number of their helpline is given on the Information Leaflet.

Key points to be covered in discussion with parents

1. While preliminary results of studies of probiotics are encouraging, particularly in respect of reducing the incidence of NEC, the studies have been relatively small and we need bigger studies to be confident about whether or not probiotics are helpful.
2. In general probiotics are thought to be a very safe treatment. However the babies being recruited into this study are more preterm and younger than babies in other studies and at higher risk of complications like NEC; we need to be confident that it really is safe to give probiotics to this age group.
3. Neither the medical and nursing staff nor the parents will know whether the baby is receiving probiotic or placebo.
4. Parents may have heard or read about probiotics and be keen to give them to their baby. It is important to explain that the probiotic we are using in the study is different from those available commercially and that unlike them it is manufactured to a very high specification and that it contains a single probiotic bacterium so that we know exactly what the baby is receiving. Although a few neonatal units do sometimes use probiotics we do not believe that any of the products being used is manufactured to this high specification and during the course of the trial we would strongly discourage the use of any other probiotic product.
5. In order that the probiotic and placebo look identical the powders are resuspended in 1/8 strength Neocate, the baby receives 1ml of the intervention each day. It is important to explain this to parents, some of whom will have been advised that their baby is at such high risk of gut complications that feeds will be withheld. They should be reassured that the choice of Neocate has been made after wide consultation. Neocate is a synthetic product designed specifically to be tolerated by babies with compromised gut; it is inconceivable that 1ml of this very dilute product could cause NEC. Furthermore parents should be advised that this will not reduce the possibility of successful later breast feeding.
6. The objective will be to continue the intervention until 36 weeks post menstrual age even if the baby is transferred to another hospital.
7. The doctors looking after the baby will decide whether a dose of intervention should be withheld. In general we believe it is safe to give the intervention even on days when the baby is unwell – the only clear contraindication is the presence of intestinal perforation.
8. We will aim to collect 2 stool samples, one as close as possible to 2 weeks post-natal and one at 36 weeks post-menstrual age. These samples will be sent to the laboratory at the Royal London Hospital and analysed to check what bacteria are in the gut. We are requesting that these samples are retained for further investigations of the mechanisms of action of probiotics. This is described in the Parent Information Leaflet and specifically asked for by question 3 on the Consent Form - if the parents object to retention of the stool samples the baby can still be recruited and take full part in the trial.
9. No other samples are collected from participating babies.
10. It should be explained and is clearly documented in the Parent Information Leaflet that the investigators will extract data from the written case notes, from the electronic data held about the baby (e.g. SEND) and that details of results of microbiological investigations will be obtained directly from the hospital laboratory.
11. Parents can withdraw their baby from the study at any time and do not have to give an explanation. If this happens we would request that we can nonetheless use the baby's clinical data and collect outstanding stool samples.
12. It will not be possible to identify any individual baby in any presentation or report arising from the trial.

Important points to remember

- Parents who do not speak English should only be approached if an appropriate adult interpreter is available.
- Consent must be obtained before logging on to the Randomisation Website.
- Having gained consent the white copy is to be sent to the PiPS Trial Office (using the FREEPOST envelopes provided), the green copy is to be put into the Data Collection File, the yellow copy is to be given to the Parent and the green copy should be put into the babies medical notes with a Parent Information Leaflet.
- In the case of twins or triplets each infant must have a separate signed consent form. It should be explained to parents that the babies will be randomised as individuals; thus siblings may be in different arms of the trial.
- In the days following recruitment, and occasionally during the baby's stay, the clinical staff should confirm informally with the parents that they understand that their baby is in the trial and that they continue to consent to this and understand the trial design.

Recruitment of babies into multiple studies

There is no theoretical reason why babies should not be actively involved with other trials or non-intervention studies while participating in the PiPS trial. If investigators have any concerns they should contact the PiPS trial office who, if not able to answer the query immediately, will contact the Chief Investigator, Professor Kate Costeloe or designated deputy for advice.

Checklist

- ✓ Has the parent(s) had an opportunity to read the Parent Information Leaflet?
- ✓ Have you explained, and has the parent(s) understood, the aim of PiPS?
- ✓ Have you explained what the trial entails - proposed treatment and description of procedures?
- ✓ Have you explained the potential benefits and potential risks of taking part in PiPS?
- ✓ Have you explained what a placebo is?
- ✓ Have you explained what a 'randomised controlled trial' is?
- ✓ Have you explained that if the parent(s) decline, the baby's care is unaffected?
- ✓ Have you explained that the parents are free to withdraw their baby (i) at any time, (ii) without having to give a reason and (iii) without affecting their baby's medical care?
- ✓ Have you told the parent(s) that their GP will be informed of their baby's participation in PiPS?
- ✓ Has there been enough opportunity for the parent(s) to ask questions?

Appendix 6 Form 6: intervention discontinuation or trial withdrawal

PiPS
Probiotic in Preterm babies Study

Form 6: Intervention Discontinuation or Trial Withdrawal

Please complete in black ballpoint pen

Please complete this form in the following circumstances:

1. If the parents wish to stop the trial intervention
2. If the parents wish to stop their baby's ongoing participation in the trial after the trial intervention has completed
3. If the baby is withdrawn from the trial for any other reason*

* Recommendations by the clinical team to discontinue the trial intervention should not usually lead to withdrawal of the baby from the trial - details of the discontinuation should be entered in question **B12** on the Baby Transfer, Discharge or Death Form (Form 3). In this circumstance Form 6 does not need to be completed.

Points to remember when completing this form:

- A parent has the **right to withdraw** their baby from the trial at any time and for any reason, without prejudice to the baby's care. They are not obliged to provide a reason for their change of mind
- **Please clarify** with the parents whether, despite stopping the intervention, they would agree for data collection to continue and for any outstanding stool samples (due at 2 weeks post-natal and 36 weeks post-menstrual age) to be collected
- If the baby is withdrawn due to an adverse event, the investigator should determine whether a **Form 5** needs to be completed and arrange for follow-up until the adverse event has resolved
- Do not forget to cancel the prescription for the trial intervention on the baby's drug chart and record the date the intervention was permanently discontinued in question **B1** of this form
- Apply a 'Course Finished' label from section 20 of the PiPS Documentation Box over the broken silver security tab on the front of the package and retain the package for a PiPS research nurse to collect (see Guidance Sheet 4)
- When this form has been completed **make a copy**, return the original to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box and place the copy in the baby's clinical notes
- Please ensure all questions on this form are answered; this will avoid unnecessary work in chasing missing data
- If you have any questions about this form or how to answer any of the questions please contact the Trial Office on [redacted]

Part A: Baby details

A1. Name of hospital: _____

A2. Study number (5 digits): | | | | | |

A3. Date of birth: D D / M M / Y Y

A4. Baby's surname: _____ **First name:** *(if known)* _____

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 Discontinuation - Withdrawal Form_v 3.0 dated 20Jun2011

Part B: Details

B1. **Date of discontinuation of intervention or withdrawal from trial:** D D / M M / Y Y

B2. **Why was the intervention discontinued, or the baby withdrawn?**
(Please tick one of the following)

Parental wish ☐
Other ☐

Please list any further information:

B3. **Have the parents agreed that the data already collected can be used?** Yes ☐ No ☐

B4. **Have the parents agreed that we can continue to collect clinical data until the baby is discharged from hospital?** Yes ☐ No ☐

B5. **Have the parents agreed that we may collect any outstanding stools (due at 2 weeks post-natal and 36 weeks post-menstrual age)?** Yes ☐ No ☐

Part C: Details of person completing form

Name of Principal Investigator or consultant or PiPS research nurse completing this form

C1. **Date this form was completed:** D D / M M / Y Y

C2. **Name of person completing this form:**

Name: *(Print)* _____ Signature: _____

C3. **What is the best way of contacting you?**

What to do now

Please cancel the prescription for the PiPS trial intervention on the baby's drug chart, apply a 'Course Finished' label to the broken security tab and retain the package for a PiPS research nurse to collect.

When this form is complete, please place a copy in the baby's clinical notes and return the original to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box.

Appendix 7 Form 1: trial entry

PiPS
Probiotic in Preterm babies Study

Form 1: Trial Entry

Please complete in black ballpoint pen

The eligibility (inclusion) criteria for PiPS are:

- Gestational age between or equal to **23 weeks and 0 days** and **30 weeks and 6 days** by the best assessment **and**
- Less than **48 hours** old **and**
- Parental written informed consent obtained

The exclusion criteria for PiPS are:

- A lethal congenital abnormality known at trial entry* **or**
- Any known gastro-intestinal malformation* **or**
- No realistic chance of survival

* Any baby discovered later to have a potentially lethal or a gastro-intestinal malformation may remain in the trial, at the discretion of parents and clinicians

Babies receiving antibiotics from birth for suspected or proven infection are eligible for the trial.

Points to remember when completing this form:

- Until complete keep this form in the 'Working Documents' section of the PiPS Documentation Box
- This Form must be completed **within 7 days** of birth and returned to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box
- **Please remember** to complete the '**Trial Participant Log**' in the PiPS Data Collection file and apply the '**PiPS Intervention Schedule**' label to the allocated package noting when the last dose should be given.
- If you make a mistake when filling out this form, strike through once and initial and date the correction (please do not use Tipp-ex!)
- Please ensure all questions on this form are answered, this will avoid unnecessary work in chasing missing data
- If you have any questions about this form or how to answer any of the questions please contact the Trial Office on [redacted]

Part A: Eligibility

A1. What was the expected date of delivery (EDD)?
(best estimate, derived from first ultrasound dating scan if possible) D D / M M / Y Y

A2. What was the actual date and time of birth? D D / M M / Y Y h h : m m (24hr)

A3. What is the baby's sex? Male ☐ Female ☐ Indeterminate ☐

A4. Is the baby a singleton or multiple birth? Singleton ☐ Multiple ☐

A5. Do you have written parental consent for the baby's participation? Yes ☐ No ☐

A6. Name of person completing Part A of the form:

Name *(Print)*: _____ Signature: _____

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 Trial Entry Form_v 3.0 dated 20Jun2011

Part B: Randomisation

At randomisation you will be given the baby's 5 digit 'Study Number' and a 'Package ID Number' for the package of trial intervention allocated for this baby; this number will have 4 digits preceded by a letter. **The package should be available on the Neonatal Unit.**

B1. Study number (5 digits): ☐☐☐☐☐

B2. Trial intervention Package ID Number (1 letter + 4 digits): ☐☐☐☐☐

As soon as you have identified the correct package you should **immediately** write the baby's

i. Name
ii. Date of Birth
iii. Study Number

on the outside of the package in the space provided and apply the '**PiPS Intervention Schedule**' label attached to this page to the front of the allocated package below the silver security tab (see Guidance Sheet 2) noting when the last dose should be given using the information from the randomisation printout. **Then prescribe** the intervention on the baby's drug chart using the wording:

'PiPS intervention nnnnn, 1ml daily, within 3hrs of preparation'

The package ID number 'nnnnn' (1 letter + 4 digits) **must** be specified on the prescription and checked with each dose of the intervention.

Inside the package are foil sachets containing the intervention as freeze dried powder. Each sachet is identified with the same number as the package. The first dose should be given as soon as possible and directions for preparing and administering the intervention are given in Guidance Sheet 3.

B3. Date the first dose of PiPS trial intervention was given: D D / M M / Y Y

B4. Time the first dose of PiPS trial intervention was given: h h : m m (24hr)

B5. Name of person completing Part B of the form:

Name *(Print):* _____ Signature: _____

Part C: Maternal and obstetric details

Please complete the remainder of this form as soon as you can and return to the PiPS Trial Office **within a week of birth**.

C1. Was the baby born in this hospital? Yes ☐ No ☐

If No, where was the baby born? _____

C2. Mother's surname: _____ **First name:** _____

C3. Mother's date of birth: D D / M M / Y Y

C4. Mother's NHS number: *(if known)* ☐☐☐☐☐☐☐☐☐☐

C5. What was the mother's full postcode at the time of the baby's birth? ☐☐☐☐☐☐☐

C6. What is the mother's ethnic group? *(please tick)*

White - British/Irish ☐ 1	Mixed - White and Asian ☐ 5	Chinese ☐ 11
White - Other ☐ 2	Mixed - Other ☐ 6	Any other ethnic category ☐ 12
Mixed - White and Black Caribbean ☐ 3	Asian - Indian ☐ 7	Black - Caribbean ☐ 13
Mixed - White and Black African ☐ 4	Asian - Pakistani ☐ 8	Black - African ☐ 14
	Asian - Bangladeshi ☐ 9	Black - Other ☐ 15
	Asian - Other ☐ 10	Not known ☐ 16

C7. **Was the mother given any ante-natal steroid to improve lung maturation?**

- No ☐
- Yes, started less than 24h before birth ☐
- Yes, started 24 or more hours before birth ☐
- Unknown ☐

C8. **Did the membranes rupture more than 24h before birth?** Yes ☐ No ☐ Unknown ☐

C9. **Was a clinical diagnosis of chorioamnionitis made in the 24h before birth?**

Yes ☐ No ☐ Unknown ☐

C10. **Did the mother receive antibiotics in the 24h before birth?** Yes ☐ No ☐ Unknown ☐

If Yes, please list all antibiotics given:

i. _____

ii. _____

iii. _____

iv. _____

Part D: Neonatal details

D1. **Baby's surname:** _____ **First name:** *(if known)* _____

D2. **Baby's NHS number:** ☐☐☐☐☐☐☐☐☐☐

D3. **Baby's hospital number in this hospital:** ☐☐☐☐☐☐☐☐☐☐

D4. **What was the baby's mode of delivery?** *(please only tick one of the following)*

- Vaginal birth – cephalic ☐
- Vaginal birth – breech ☐
- Vaginal birth – other presentation ☐
  - **If Other**, please specify _____
- Caesarean section before onset of labour ☐
- Caesarean section after onset of labour ☐

D5. **Were forceps or Ventouse used to effect delivery?** Yes ☐ No ☐

D6. **What was the main cause of the preterm birth?** *(please only tick one of the following)*

- Prelabour rupture of membranes (PPROM) ☐
- Preterm labour (without PROM) ☐
- APH ☐
- PIH (+/- APH) ☐
- Other maternal illness* ☐
- Poor fetal growth (mother well) ☐

***Other maternal illness:** Any pregnancy where the main reason for preterm delivery was a maternal problem such as infection, renal disease or pre-pregnancy diabetes, hypertension or trauma.

D7. **What was the baby's birthweight?** ☐☐☐☐ g

D8. **Was the baby one of a multiple pregnancy?** Yes ☐ No ☐

If Yes, how many babies were born? ☐

What was the birth order of this baby? ☐

D9. **Was the baby's heart rate >100bpm at 5 minutes of age?** Yes ☐ No ☐

D10. **What was the baby's temperature when first admitted to the neonatal unit?** ☐☐.☐ °C

D11. **What was the Apgar score at 5 minutes?** ☐☐

D12. **What was the baby's worst base excess in the first hour after birth?** ☐☐.☐

Part E: Hospitals to which this baby may be transferred

We aim to continue the intervention until 36 weeks post-menstrual age and data collection until discharge. Many babies in the trial are likely to be transferred to a hospital nearer home before 36 weeks. In order to complete the intervention and data collection the PiPS trial will need to have R&D approval in that hospital. We are therefore asking you to tell us where the baby might be transferred to so we can confirm that we have the necessary authorisations and have provided training at that hospital.

E1. **Is this baby likely to be transferred to another hospital before discharge home?** Yes ☐ No ☐

If Yes, please list the names of the hospitals to which the baby is most likely to go:

i. _____

ii. _____

iii. _____

iv. _____

Part F: Details of the person completing this form

F1. **Date this form was completed** D D / M M / Y Y

F2. **Name of person completing this section of the form:**

Name *(Print)*: _____ Signature: _____

F3. **Name of hospital:** _____

F4. **What is the best way of contacting you?**

When this form is complete

Please return to the PiPS Trial Office using a PiPS FREEPOST envelope within **7 days of birth**.

Appendix 8 Form 2: daily data

Example

Study number: 1 2 3 4 5 — Please start on day of birth (day 1) and continue until the final column is completed. — Study number: 1 2 3 4 5

Part C: Enteral Feed Details

Please tick each type of milk given on each day and give the total daily volume in ml/kg.

Please collect the first stool sample on day 14 or as near as possible to this date: →
When was this stool sample sent off? 3 1 / 0 7

Date (dd/mm - add to top row)	D.O.B.	18/07	19/07	20/07	21/07	22/07	23/07	24/07	25/07	26/07	27/07	28/07	29/07	30/07
No milk given	☑	☐	☐	☐	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐
Expressed maternal milk	☐	☑	☑	☐	☐	☐	☑	☑	☑	☑	☑	☑	☑	☐
Donor breast milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Preterm formula *(see Part B)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Term formula *(see Part B)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Total milk ml/kg/day		0 0 2	0 0 8	0 0 4			0 0 7	0 2 6	0 5 8	0 7 3	1 2 9	1 6 6	1 1 0	
Fed directly from the breast	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☑	☑

Part D: Antibiotics and Antifungals

Please tick each day that systemic antibiotics AND/OR antifungals are given. Please include antibiotics or antifungals given for prophylaxis.

Any penicillin (Benzyl pen, Fluclox, Amp etc.)	☑	☑	☑	☑	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any aminoglycoside (Gentamicin, Amikacin etc.)	☑	☑	☑	☑	☐	☐	☑	☑	☑	☐	☐	☐	☐	☐
Any cephalosporin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any glycopeptide (Vancomycin, Teicoplanin etc.)	☐	☐	☐	☑	☑	☑	☑	☑	☑	☐	☐	☐	☐	☐
Any carbapenem (Imipenem, Meropenem etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any β lactam / inhibitor (Tazocin, Co-amoxiclav etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any macrolide (Erythromycin, Clarithromycin etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other antibiotic (please specify)														
Prophylactic antifungal	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑	☑
Antifungal to treat suspected or proven infection	☐	☐	☐	☐	☐	☐	☑	☑	☑	☑	☑	☑	☑	☑

Part E: Antacids

Please tick each day that systemic ranitidine / proton pump inhibitor are given.

Ranitidine or proton pump inhibitor (e.g. Omeprazole)	☐	☐	☐	☐	☐	☑	☑	☑	☑	☑	☐	☐	☐	☐

PiPS — Probiotic in Preterm babies Study

Form 2: Daily Data Collection

Please complete in black ballpoint pen

Points to remember when completing this form:

- If the baby is transferred before this form is completed (i.e. **if less than 14 days post-natal age**), that the partially completed form is copied and the original is transferred with the baby (the copy is to be filed in the PiPS Data Collection File)
- If you are unable to photocopy the whole A3 sheet then copy each side onto A4 paper so that you have 4 pages ensuring the Study Number is on each page
- When this form has been completed, return to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box
- If you make a mistake when filling out this form, strike through once and initial and date the correction (please do not use Tipp-ex!)
- Please ensure all questions are answered and that you record as much information as possible, this will avoid unnecessary work in chasing missing data
- An example of how this form should be filled out is given on the back page
- If you have any questions about this form or how to answer any of the questions please contact the Trial Office on [redacted]

Part A: Baby details

Name of hospital: _____

Baby's surname: _____ **First name:** *(if known)* _____

Study number (5 digits): ☐☐☐☐☐ **Date of birth:** D D / M M / Y Y

Part B: Proprietary milk formulas

Please list below the names of all proprietary milk formulas the baby has received during the first two weeks after birth: *(This information is needed because some of these contain prebiotic which might influence probiotic colonisation)*

npeu National Perinatal Epidemiology Unit — Queen Mary University of London — University of Oxford

Study number: ☐☐☐☐☐ Please start on day of birth (day 1) and continue until the final column is completed. **Study number:** ☐☐☐☐☐

Part C: Enteral Feed Details

Please tick each type of milk given on each day and give the total daily volume in ml/kg.

Please collect the first **stool sample on day 14** or as near as possible to this date: →

When was this stool sample sent off? D D / M M

Date *(dd/mm - add to top row)*	D.O.B.	/	/	/	/	/	/	/	/	/	/	/	/	/
No milk given	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Expressed maternal milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Donor breast milk	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Preterm formula *(see Part B)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Term formula *(see Part B)*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Total milk ml/kg/day	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐	☐☐☐
Fed directly from the breast	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Part D: Antibiotics and Antifungals

Please tick each day that systemic antibiotics **AND/OR** antifungals are given. Please include antibiotics or antifungals given for prophylaxis.

Any penicillin (Benzyl pen, Fluclox, Amp etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any aminoglycoside (Gentamicin, Amikacin etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any cephalosporin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any glycopeptide (Vancomycin, Teicoplanin etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any carbapenem (Imipenem, Meropenem etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any β lactam / inhibitor (Tazocin, Co-amoxiclav etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Any macrolide (Erythromycin, Clarithromycin etc.)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other antibiotic (please specify)														
Prophylactic antifungal	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Antifungal to treat suspected or proven infection	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Part E: Antacids

Please tick each day that systemic ranitidine / proton pump inhibitor are given.

Ranitidine or proton pump inhibitor (e.g. Omeprazole)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Appendix 9 Form 3: transfer–discharge

PiPS
Probiotic in Preterm babies Study

Form 3: Baby Transfer, Discharge or Death

Outcomes at this hospital

Points to remember when completing this form:

- This form should be completed at **discharge** whether to **another hospital** or to **home**, or at **death**
- If the baby is discharged to another hospital please remember to complete the **PiPS Transfer Checklist** (see Guidance Sheet 6) and inform the Trial Office of the transfer on [redacted]
- If the baby is being transferred and is below 36 weeks post-menstrual age, please also send the baby's allocated package with all the unused sachets of the intervention to the receiving hospital in the Transfer Pack
- **Only events** occurring in this hospital should be recorded on this form; a similar form will be completed in each hospital where the baby is admitted
- If the baby reached 36 weeks pma or was discharged home or died before this date while at your hospital please ensure you complete **Part C** of this form
- Until completed, keep this form in the 'Working Documents' section of the PiPS Documentation Box
- When this form has been completed, return to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box **with all Form 4: Abdominal Pathology** forms which have been completed for episodes of any suspected abdominal pathology while the baby was an in-patient at your hospital
- If you make a mistake when filling out this form, strike through once and initial and date the correction (please do not use Tipp-ex!)
- Please ensure all questions on this form are answered; this will avoid unnecessary work in chasing missing data
- If you have any questions about this form or how to answer any of the questions please contact the Trial Office on [redacted]

Part A: Baby details

A1. Name of hospital: _____

A2. Study number (5 digits): ☐☐☐☐☐

A3. Date of birth: D D / M M / Y Y

A4. Baby's surname: _____ **First name:** *(if known)* _____

A5. Baby's NHS number: ☐☐☐☐☐☐☐☐☐☐

A6. Baby's hospital number in this hospital: ☐☐☐☐☐☐☐☐☐☐

A7. Date of admission to this hospital*: D D / M M / Y Y

If at admission to this hospital the baby is over 36 weeks post-menstrual age you **do not need to complete **Part C** of this form.*

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 Discharge or Transfer Form_v 4.0 dated 20Jun2011

Part B: While in this hospital

Infections

B1. **While in this hospital were there any episodes of NEC or other abdominal pathology?** Yes ☐ No ☐

If Yes, how many episodes: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐

*A separate Abdominal Pathology form (**Form 4**) should be completed for each episode and submitted to the PiPS Trial Office with this form.*

B2. **While in this hospital did you grow any bacteria from a normally sterile site other than blood or CSF:** *e.g. SPA, intra-operative peritoneal swab, abscess drainage etc.* Yes ☐ No ☐

If Yes, please complete table below *(blood and CSF culture data are being obtained directly from the microbiology laboratory, please don't enter here):*

Date sample taken	Sample site (e.g SPA, intra-operative swab, etc.)
D D / M M / Y Y	
D D / M M / Y Y	
D D / M M / Y Y	
D D / M M / Y Y	
D D / M M / Y Y	

Feeding

B3. **While in this hospital did the baby reach full feeds (150 ml/kg/day) for the first time?** Yes ☐ No ☐

If Yes, what was the date that the baby first reached 150 ml/kg/day of milk? D D / M M / Y Y

Note: If the baby was breast feeding before receiving 150ml/kg/day by tube please count the first day that IV fluid supplements were discontinued as the day full feeds were achieved.

(Fluid supplements should include any fluid e.g TPN or dextrose solution given as part of the baby's total fluid prescription but not fluid given solely to administer iv medications)

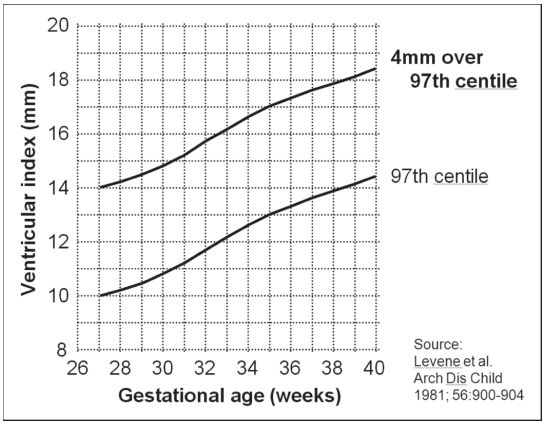
Other diagnoses

B4. While in this hospital did the baby have any cerebral ultrasound scans? Yes ☐ No ☐

If Yes, identify below any abnormalities seen on any scan in this hospital *(please tick at least one box in each column)*:

Please select from the following:	Left	Right
No abnormality seen on any scan	☐	☐
Intraventricular haemorrhage (IVH)	☐	☐
Haemorrhagic parenchymal infarct (HPI)	☐	☐
Hydrocephalus (Ventricular index >4mm above 97th centile*)	☐	☐
Porencephalic cyst	☐	☐
Periventricular leucomalacia (PVL)	☐	☐

*Ventricular Index: The ventricular index is the distance between the middle and the most lateral point of the lateral ventricle in millimetres measured in the coronal (or the axial) plane at the level of the foramen Munro.



B5. While in this hospital did the baby receive treatment for Patent Ductus Arteriosus? Yes ☐ No ☐

If Yes, please indicate treatment:

Medical treatment with indometacin or ibuprofen ☐

Surgical ligation ☐

B6. While in this hospital were any congenital malformations detected? Yes ☐ No ☐

If Yes, please list congenital malformations below

B7. **While in this hospital were the eyes examined for Retinopathy of Prematurity?** Yes ☐ No ☐

If Yes, is any ROP present? Yes ☐ No ☐

If Yes, what was the worst stage of ROP in each eye?
(See stage definitions below. Please enter '0' if not present) Right Eye ☐ Left Eye ☐

Has the ROP been treated with laser or cryotherapy? Yes ☐ No ☐

If Yes: Right Eye ☐ Left Eye ☐

Definitions of stage of ROP *(Arch Opthalmol 2005;123:991-9)*:

Stage 1: Demarcation line - A thin relatively flat line separating the vascular and avascular retina. Abnormal branching or arcading of vessels may lead up to the demarcation line.

Stage 2: Ridge - The ridge has height and width extending above the retina. Isolated tufts of neovascular tissue - "popcorn" - may be seen posterior to the ridge.

Stage 3: Extraretinal Fibrovascular Proliferation - In this stage extraretinal fibrovascular proliferation or neovascularisation extends from the ridge into the vitreous.

Stage 4: Partial Retinal Detachment - Sparing macula (stage 4a) and involving macula (stage 4b).

Stage 5: Total Retinal Detachment

B8. **While in this hospital did the baby receive any antimicrobials (antibiotics or antifungals) for suspected or proven infection after 14 days post-natal age?** Yes ☐ No ☐

If Yes, please specify:

For how many days in total were antibiotics given? ☐☐☐ days

For how many days in total were antifungals given? ☐☐☐ days

(Antimicrobials used up until 14 days after birth are reported on Form 2, the Daily Data Collection form)

Please do not include days of antimicrobials given for prophylactic use e.g. prophylactic peri-operative antibiotics or prophylactic nystatin or fluconazole.

B9. **While in this hospital were any surgical procedures performed other than for duct ligation, NEC or other abdominal pathology?** Yes ☐ No ☐

If Yes, please identify procedure:

Repair of inguinal hernia ☐

Insertion of ventricular reservoir ☐

Insertion of ventriculo-peritoneal shunt ☐

Other *please specify* _____

Any abdominal surgery should be recorded separately on ***Form 4****, the Abdominal Pathology form.*

Intensive / high dependency care

See definitions on back page

B10. While in this hospital, what was the total number of days of intensive / high dependency care?

Level 1 (intensive care) ☐☐☐ days

Level 2 (high-dependency care) ☐☐☐ days

B11. While in this hospital what was the total number of days for which the baby had a central venous line (UVC, peripheral long line, Broviac etc.) ☐☐☐ days

Please do not leave date fields blank e.g. if a baby does not receive any Level 1 care please indicate as '0' days.

Trial intervention

B12. While in this hospital was the trial intervention discontinued before 36 weeks post-menstrual age for any reason other than discharge from hospital? Yes ☐ No ☐

If Yes, was the trial intervention discontinuation:

Temporary Yes ☐ No ☐

If Yes, for how many days in total was the trial intervention discontinued: ☐☐ days

Please specify reason:

Permanent Yes ☐ No ☐

If Yes, why was the trial intervention discontinued?

Please specify the date the intervention was discontinued: D D / M M / Y Y

i) Parental request Yes ☐ No ☐

*(if the baby is withdrawn at parental request please complete **Form 6** and notify the PiPS trial office as soon as possible)*

ii) Clinician recommendation Yes ☐ No ☐

Please specify reason if known:

*(if the intervention was discontinued because of an SAE please complete a **Form 5**)*

B13. While in this hospital was the baby in any other trial? Yes ☐ No ☐

If Yes, please give the trial name(s) _____

Part C: Information at 36 weeks and 0 days post-menstrual age or discharge or death if sooner

C1. While in this hospital *(Please tick one of the following options):*

i. Was the baby transferred to another hospital before 36w pma? **If Yes**, ☐ go to Part D.

ii. Did the baby reach 36 weeks pma? **If Yes**, ☐ complete the rest of Part C.

iii. Was the baby discharged home or did the baby die before 36 weeks pma? **If Yes**, ☐ complete the rest of Part C*.

**If the baby was discharged home or died before 36 weeks post-menstrual age please complete the questions below using data available as close as possible to the date of discharge or death.*

C2. What was the date the baby reached 36 weeks pma or was discharged home or died if sooner? D D / M M / Y Y

On this date:

i. Was the baby still receiving any mechanical respiratory support including nCPAP or via a humidified high flow device e.g. Vapotherm delivering ≥2l/min? Yes ☐ No ☐

ii. Had the baby been given post-natal corticosteroids at any time with the intention to reduce the severity of Bronchopulmonary Dysplasia? Yes ☐ No ☐

iii. Was the baby receiving supplementary oxygen? Yes ☐ No ☐

*If prior to **this date** the baby has been stable in air and on this date the baby goes **briefly** into oxygen for an event such as a hernia repair or ROP treatment please answer 'No'*

If Yes,

Was the oxygen <30% ☐ **or** ≥30% ☐

Or if the oxygen was given by nasal cannulae

Was the oxygen ≤0.1l/min ☐ **or** >0.1l/min ☐

C3. As close as possible to the date the baby reached 36w or was discharged home or death:

i. What was the baby's weight ☐☐☐☐ g Date of measurement D D / M M / Y Y

ii. What was the baby's OFC ☐☐.☐ cm Date of measurement D D / M M / Y Y

C4. Was a stool sample collected as close as possible to 36 weeks pma?* Yes ☐ No ☐

If Yes, when was it sent off? *(if known)* D D / M M / Y Y

**If the baby was discharged home before 36 weeks a stool sample should be collected as close as possible to discharge (see stool collection step by step guide in the Guidance Sheet booklet).*

C5. What was the last day the trial intervention was given? D D / M M / Y Y

At 36 weeks and 0 days pma or discharge home or death, if sooner, the remaining sachets should be retained in the allocated package. A 'Course Finished' label from section 20 of the PiPS Documentation Box should be applied over the broken silver security tab on the front of the package and it should be retained for a PiPS research nurse to collect (see Guidance Sheet 4).

Part D: Baby's outcome

Baby's outcome in this hospital *(Please complete only one of the following questions - D1, D2 or D3)*

D1. Discharged home: Date of discharge D D / M M / Y Y

D2. Transferred to another hospital: Date of transfer D D / M M / Y Y

Name, address and telephone number of receiving hospital:

Name of receiving consultant *(if known)*

D3. Death: Date of death D D / M M / Y Y

i. Is a post-mortem examination planned or already performed? Yes ☐ No ☐

ii. What do you consider the principal cause of death *(Please only tick one of the following options)*

Respiratory failure	☐	Congenital malformation	☐
Brain injury	☐	Infection	☐
NEC	☐	Other gut pathology	☐

Other please specify

iii. Was intensive care actively withdrawn? Yes ☐ No ☐

If the baby died, please send a copy of the discharge summary and, if available, the post-mortem report to the PiPS Trial Office.

Part E: Contact details *Please provide as much detail as possible*

Mother:
First Name:
Surname:
Address:
Telephone:
Mobile:
Email:

Father:
First Name:
Surname:
Address:
Telephone:
Mobile:
Email:

Family Doctor:
First Name:
Surname:
Address:
Telephone:
Email:

Paediatrician responsible for follow up:
First Name:
Surname:
Address:
Telephone:
Email:

Part F: Details of the person completing this form

F1. Date this form was completed D D / M M / Y Y

F2. Name of person completing this form

Name *(Print)*: _____ Signature: _____

F3. What is the best way of contacting you?

When this form is complete

When this form is complete return with all Abdominal Pathology Forms (Form 4) which have been completed for episodes of any suspected abdominal pathology while the baby was an in-patient at your hospital to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box

Definitions for intensive / high dependency care

Intensive care includes babies:

Receiving any respiratory support via an endotracheal tube and in the first 24 hours after its withdrawal

Receiving nCPAP for any part of the day and less than five days old

Below 1000g current weight and receiving nCPAP for any part of the day and for 24 hours after withdrawal

Less than 29 weeks' gestational age and less than 48 hours old

Requiring major emergency surgery, for the pre-operative period and post-operatively for 24 hours

Requiring complex clinical procedures:

- Full exchange transfusion
- Peritoneal dialysis
- Infusion of an inotrope, pulmonary vasodilator or prostaglandin and for 24 hours afterwards

Any other very unstable baby considered by the nurse-in-charge to need 1:1 nursing

A baby on the day of death

High dependency cares includes babies:

Receiving nCPAP for any part of the day and not fulfilling any of the criteria for intensive care

Below 1000g current weight and not fulfilling any of the criteria for intensive care

Requiring parenteral nutrition

Having convulsions

Receiving oxygen therapy and below 1500g current weight

Requiring treatment for neonatal abstinence syndrome

Requiring specified procedures that do not fulfil any criteria for intensive care:

- Care of an intra-arterial catheter or chest drain
- Partial exchange transfusion
- Tracheostomy care until supervised by the parent

Requiring frequent stimulation for severe apnoea

npeu National Perinatal Epidemiology Unit

Queen Mary University of London

University of Oxford

Appendix 10 Form 4: abdominal pathology

PiPS
Probiotic in Preterm babies Study

Form 4: Abdominal Pathology

Please complete in black ballpoint pen

Points to remember when completing this form:

- Please **complete a separate** Form 4 for any episode of proven or suspected abdominal pathology including necrotising enterocolitis while the baby is an in-patient at your hospital (modified Bell criteria for staging NEC overleaf)
- Keep completed and 'in progress' forms in the 'Working Documents' section of the PiPS Documentation Box
- **All completed** Abdominal Pathology forms should be returned at transfer, discharge or at death with the completed Form 3 to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box
- Please ensure all questions on this form are answered; this will avoid unnecessary work in chasing missing data. If you have any questions please contact the Trial Office on [redacted]

Part A: Baby details

Name of hospital: _____

Baby's surname: _____ **First name:** *(if known)* _____

Study number (5 digits): ☐☐☐☐☐ **Date of birth:** D D / M M / Y Y

Part B: Episode details

B1. Date episode started D D / M M / Y Y

B2. What was the final diagnosis? *(Please tick one of the following)*

i. Necrotising enterocolitis *(please select stage)*

- Suspected NEC not fulfilling criteria for Stage II ☐
- Stage II A or B: Definite NEC ☐
- Stage III A: Advanced NEC, no perforation ☐
- Stage III B: Advanced NEC with perforation ☐

ii. Isolated intestinal perforation, no NEC ☐

iii. Septic ileus ☐

iv. Meconium or milk plug ☐

v. Other, please specify _____

B3. Was there definite pneumotosis intestinalis (intra-mural gas) at any time? Yes ☐ No ☐

B4. Was there intra-hepatic gas at any time? Yes ☐ No ☐

B5. Was there intestinal perforation at any time? Yes ☐ No ☐

B6. Did the baby have surgery in association with this episode? *(Please tick all that apply)*

No ☐ Peritoneal drainage alone ☐

Laparotomy, no enterostomy ☐ Laparotomy, with enterostomy ☐

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 Abdominal Pathology Form_v 3.0 dated 20Jun2011

Part C: Details of person completing form

Name of Principal Investigator or consultant or PiPS research nurse completing this form

C1. Date this form was completed D D / M M / Y Y

C2. Name of person completing this form

Name: *(Print)* _____ Signature: _____

C3. What is the best way of contacting you?

Modified Bell criteria for staging NEC

Bell stage Signs	Systemic	Gastro-intestinal	Radiographic
Stage IIA (*Definite NEC: mildly ill)*	Increased desaturations and/or bradycardia Temperature instability Lethargy	Increased pre-feed gastric aspirate Definite abdominal distension Possible abdominal tenderness Possibly bloody stools	Definite abdominal dilatation Pneumotosis intestinalis
Stage IIB *(Definite NEC: moderately ill)*	As IIA with platelets <100 x 1012 and/or metabolic acidosis: base excess <-8meq/l	Abdominal distension with definite tenderness Possible abdominal wall oedema and/or erythema	As **IIA** with portal vein gas Possible ascites
Stage IIIA *(Advanced NEC: bowel intact)*	As **IIB** plus mixed acidosis: pH <7.2 DIC Neutropaenia <1x10^9/l Severe apnoea Hypotension requiring inotropes	Generalised peritonitis with severe tenderness with abdominal wall induration	As **IIA** with definite ascites
Stage IIIB *(Advanced NEC: bowel perforated)*	As **IIIA**	As **IIIA**	As **IIIA** with pneumoperitoneum

Reference

1. Walsh MC, Kliegman RM. Necrotising enterocolitis: treatment based on staging criteria. Pediat Clin North Am, 1986;33:179-201

Appendix 11 Form 5: for reporting serious adverse events and suspected unexpected serious adverse reactions

PiPS
Probiotic in Preterm babies Study

Form 5: Serious Adverse Event (SAE) & Suspected Unexpected Serious Adverse Reaction (SUSAR)

Please complete in black ballpoint pen

Action required by clinician

i. Complete this form **within 24 hours** of becoming aware of the event
ii. Fax immediately to the PiPS Trial Office at the NPEU on [redacted]
iii. Make a copy of this form, send the original to the Trial Office using a FREEPOST envelope from the PiPS Documentation Box and place the copy in the baby's medical notes

Standard Operating Procedure for the reporting of unexpected Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs)

Expected SAEs: All expected SAEs will be recorded on data collection forms and will be reviewed by the Data Monitoring Committee at regular intervals throughout the trial.

These do not require to be reported on this form. In the context of this trial this includes:

- Death
- Culture positive infection with organisms other than *bifidobacteria*
- Necrotising enterocolitis or focal intestinal perforation
- Broncho-pulmonary dysplasia
- Intracranial abnormality (haemorrhage or focal white matter damage) on cranial ultrasound scan or other imaging
- Pulmonary haemorrhage
- Patent ductus arteriosus
- Retinopathy of Prematurity requiring retinal surgery

Unexpected SAEs: An unexpected serious adverse event is one that is not anticipated and is not known to be related to the condition being studied or the treatment being offered. These should be reported immediately using this form.

SUSAR: A suspected adverse reaction related to the treatment that is both serious and unexpected (i.e. not consistent with the expected outcomes of the treatment being offered). These should be reported immediately using this form.

The SUSARS that have been noted prospectively in the context of the PiPS trial are:

i. Intestinal obstruction associated with corn starch
ii. Bacteraemia with *Bifidobacterium breve* BBG

Neither of these is expected to occur, however

- If you suspect intestinal obstruction due to corn starch you should complete this form and fax it to the PiPS Trial Office within one working day at [redacted].
- Microbiology laboratories have been asked to notify the isolation of any *bifidobacterium* from a normally sterile site to Dr Michael Millar. Dr Mark Wilks or nominated deputy in the microbiology laboratory at the Royal London Hospital will be sent a sample of the isolated *bifidobacterium* organism for typing. If the organism is found to be *Bifidobacterium breve* (which can be done within one working day) the SUSAR will be reported from the microbiology laboratory and the PI at the hospital will be notified. The identification of the strain precisely as *Bifidobacterium breve* BBG make take several weeks longer. When that process is completed all parties will be notified.

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 SAE & SUSAR Form_v 2.0 dated 30Jun2010

Part A: Reporting information

A1. **Name of hospital** _____

A2. **Name of Principal Investigator or Consultant completing the form**

Surname _____ First Name _____

A3. **Date form completed:** D D / M M / Y Y

Part B: Baby identification details

B1. **Baby's surname** _____ **First name** *(if known)* _____

B2. **Study number (5 digits)**

B3. **Date of birth:** D D / M M / Y Y

Part C: Details of event

C1. **Please record the diagnosis or describe the event as briefly as possible**

C2. **Date and time event started** D D / M M / Y Y h h : m m (24hr)

C3. **Date and time event resolved (if resolved)** D D / M M / Y Y h h : m m (24hr)

C4. **Indicate the severity of the event** Mild ☐ Moderate ☐ Severe ☐

C5. **Indicate the level of causality that you consider there is between the intervention and this event** Possibly related ☐ Not related to the intervention ☐

C6. **Was this event a SUSAR?** Yes ☐ No ☐ Unsure ☐

C7. **Outcome**

Recovered ☐
Recovering ☐
Continuing ☐
Baby died ☐
Unknown ☐

C8. **Are there any clinical sequelae?** Yes ☐ No ☐ Unsure ☐

If Yes, please describe

Part D: Treatment details

D1. Was any treatment required in response to the event reported? Yes ☐ No ☐

If Yes, *please continue*

If No, *please go to* ***Part F***

D2. If specific drug therapy was prescribed for the event, please list all drugs used in the table below *(Continue on a separate sheet if necessary)*

Drug given *(generic name)*	Dosage regimen	Route of admin.	Date and time started	Date and time stopped
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr

D3. Were any non-medical therapies, e.g. surgery, provided in response to this event? Please provide details in the box below. *(Continue on a separate sheet if necessary)*

PiPS_ISRCTN No: 05511098_REC Ref: 09/H0604/30 SAE & SUSAR Form_v 2.0 dated 30Jun2010

Part E: Treatment details

E1. Please list other drugs being given at or around the time of the event *(do not include routinely used IV fluids)*.

Drug given *(generic name)*	Dosage regimen	Route of admin.	Date and time started	Date and time stopped
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr
			D D / M M / Y Y h h : m m 24hr	D D / M M / Y Y h h : m m 24hr

Part F: Further information

F1. Were any further investigations taken after becoming aware of the event? Yes ☐ No ☐

If Yes, please specify

F2. Is there any other relevant information? Yes ☐ No ☐

If Yes, please add anything else that you think we should know here

F3. As a result of this event was the trial intervention permanently discontinued? Yes ☐ No ☐

F4. As a result of this event was the baby withdrawn from the tial? Yes ☐ No ☐

If Yes, please complete Form 6.

Appendix 12 Statistical analysis plan

PiPS: Trial of probiotic administered early to prevent infection and necrotising enterocolitis

Statistical Analysis Plan

Version 1 signed – 18th February 2014

Contributors: Pollyanna Hardy (Senior Trial Statistician)~
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PiPS: Trial of probiotic administered early to prevent infection and necrotising enterocolitis (ISRCTN Number: 05511098; Eudract Number: 2006-003445-17)

TABLE OF CONTENTS

1. INTRODUCTION

This document details the proposed presentation and analyses for the main publications reporting results from the HTA funded multicentre randomised placebo controlled trial of early administration of the probiotic *Bifidobacterium breve* strain BBG (*B. breve* BBG) to preterm infants (PiPS). The results reported in these publications will follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, although they are expected to follow the broad principles described. The principles are not intended to curtail exploratory analysis (for example, to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (for example, data transformation prior to analysis), but they are intended to establish the rules that will be followed, as closely as possible, when analysing and reporting the trial.

The analysis plan will be available on request when the principal manuscripts are submitted for publication. Suggestions for subsequent analyses by journal editors or referees will be considered carefully and carried out, as far as possible, in line with the principles of this analysis plan.

Any deviations from the statistical analysis plan will be described and the rationale given in the final report of the trial. The analysis will be carried out by an identified, appropriately qualified and experienced statistician, who will ensure the integrity of the data during processing. Examples of such procedures include quality control and evaluation procedures. This document and the interim and final analyses will be produced in line with NPEU Standard Operating Procedures ST 105 Statistical Analysis Plan; ST 104 Interim Statistical Analysis; and ST 106 Final Statistical Analysis and Reporting.

1.1.1 Chief Investigator

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XXXX

1.1.2 Trial Statisticians

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1.1.6 Trial Programmers

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XXXX
XXXX

2. BACKGROUND INFORMATION

2.1 Aims of the Trial

The aims of the trial were pre-specified in the protocol and are set out here.

2.1.1 Primary aims

To evaluate if early administration of the probiotic *Bifidobacterium breve* strain BBG (*B. breve* BBG) to preterm infants compared to placebo reduces the risk of:

- late onset blood stream infection diagnosed on a sample drawn after 72 hours,
- necrotising enterocolitis,
- death before discharge from hospital.

2.1.2 Secondary aims

To evaluate the effect of early administration of the probiotic *Bifidobacterium breve* strain BBG (*B. breve* BBG) to preterm infants compared to placebo on:

- The composite outcome of any, or a combination of, the three primary outcomes
- microbiological outcomes such as blood steam infection with skin commensals, number of babies with a blood culture taken, number of blood cultures taken per baby;
- other clinical outcomes such as the use of antibiotics for treatment of infection, broncho-pulmonary dysplasia, hydrocephalus, retinopathy of prematurity, length of stay in the neonatal unit;

- nutritional and gastroenterological outcomes such as achieving full feeds and weight gain.

The presence of the probiotic intervention strain in stool samples will also be reported as a process outcome by trial arm.

2.2 Trial Design

PiPS is a multi-centre double blind randomised controlled trial of the early administration to preterm infants of the probiotic *Bifidobacterium breve* strain BBG (B. breve BBG) or placebo.

Date of start of recruitment:	July 2010
Target end date of recruitment:	July 2013
Target number of participants:	650 in each arm
Recruiting centres:	23 UK neonatal units

2.3 Eligibility

The eligibility criteria for the trial were pre-specified in the protocol and are set out here.

2.3.1 Hospital eligibility

Hospitals with neonatal units admitting around 50 babies or more each year born before 31 completed weeks of gestation (up to and including 30 weeks + 6 days) were eligible to join the study.

2.3.2 Infant eligibility

Inclusion criteria:
Babies with all of the following criteria wereeligible for recruitment to the study:

- gestational age between or equal to 23 weeks and 0 days and 30 weeks and 6 days by the best estimate of Expected Date of Delivery (usually by first trimester antenatal ultrasound, alternatively by 'certain' LMP);
- less than 48 hours old;
- with written informed parental consent.

Babies already on antibiotics for suspected or proven infection were eligible for recruitment to the study.

Exclusion criteria:
Babies with any of the following criteria were excluded from the study:

- a lethal congenital abnormality known at trial entry;
- any known gastrointestinal malformation;
- no realistic prospect of survival.

2.4 Treatment Interventions

2.4.1 Investigational medicinal product and placebo

The investigational product tested was *Bifidobacterium breve* strain BBG (*B. breve* BBG). The product was supplied freeze dried with corn starch; the placebo was corn starch alone.

2.4.2 Preparation and Dose

The freeze dried powder was suspended in 3 ml 1/8 strength (1 scoop to 240 ml sterile water) of the elemental infant formula Neocate and allowed to settle for 30 minutes.
1 ml of supernatant was withdrawn and given to the baby; for the active product this contained 6.7×10^7 - 6.7×10^9 colony forming organisms.

2.4.3 Dosing schedule

Once daily.

2.4.4 Route of administration

The products were administered via a naso-gastric or oro-gastric tube or, for babies no longer tube fed, directly into the mouth using a syringe.

2.4.5 Treatment period

Starting as soon as possible after randomisation and continued until 36 completed weeks of post-menstrual age (36 weeks + 0 days) or death or discharge from hospital if sooner. If the baby was transferred between different neonatal units, e.g. transferred back to a local unit when he/she no longer needed intensive care, where possible the intervention was continued until the course was completed.

2.5 Principal Comparisons of Interest

The comparison of primary interest is whether there is a difference in any of three primary outcomes, infection, NEC or death, between the groups of the trial. As the primary analysis is by intention-to-treat, the outcomes will be compared across randomised groups for all infants recruited regardless of whether, or for how long, they received the PiPS trial interventions.

2.6 Definition of Primary and Secondary Outcomes

2.6.1 Primary outcomes

1. Any baby with an episode of blood stream infection, with any organism other than a skin commensal, diagnosed on a sample of blood drawn more than 72 hours after birth and before 46 weeks post-menstrual age, death or discharge from hospital, whichever is soonest. Skin commensals include coagulase negative staphylococci (CoNS) and Corynebacteria (definitions in Appendix 1);

2. Necrotising enterocolitis, Bell stage II or III (definitions in Appendix 2);

3. Death before discharge from hospital.

Data will be censored at the date of the final database lock (see section 3.3). Therefore if a baby is still in hospital at this time, they will be considered alive for the purposes of defining this primary outcome.

2.6.2 Secondary outcomes

1. Number of babies with the composite outcome of any or a combination of the 3 primary outcomes.

Microbiological outcomes: (definitions in Appendix 1)

(Outcomes 2 to 7 are for samples taken more than 72 hours after birth and before 46 weeks post-menstrual age, death or discharge home, whichever is soonest)

2. Number of babies with any positive blood culture with an organism recognised as a skin commensal e.g. CoNS or Corynebacteria;

3. Number of babies with blood cultures taken;

4. Number of blood cultures taken per baby;

5. Number of babies with episodes of blood stream infection with organisms other than skin commensals by organism: e.g. *E. coli*, *Klebsiella* spp., fungi; and by antibiotic resistance types: specifically MRSA, vancomycin resistant enterococci (VRE) and extended spectrum betalactamase producing Gram negative bacteria (ESBL);

6. Number of babies with isolates of organisms other than skin commensals from a normally sterile site other than blood: e.g. CSF, supra-pubic aspiration of urine, pleural cavity etc.;

7. Number of babies with a positive culture of *B. breve* BBG from any normally sterile site;

8. Total duration of days of antibiotics and/or anti-fungals administered per baby after 72 hours and until 46 weeks post-menstrual age, death or discharge from hospital whichever is sooner for treatment of suspected or proven sepsis i.e. excluding prophylactic use;

9. The number of babies colonised with the administered probiotic strain defined by the isolation of *B. breve* BBG from stool samples at 2 weeks post-natal and at 36 weeks post-menstrual age;

10. Stool flora: the number of babies colonised with MRSA, VRE (vancomycin resistant enterococci) or extended spectrum betalactamase producing Gram

negative bacteria (ESBL) at 2 weeks post-natal and at 36 weeks postmenstrual age.

Nutritional and gastroenterological outcomes:

11. Age at achieving full enteral nutrition (defined as 150 ml/kg/day for 1 day);

12. Change of weight Z score from birth to 36 weeks post-menstrual age or discharge from hospital if sooner.

Other clinical outcomes:

13. Broncho-pulmonary dysplasia: (definitions in Appendix 3);

14. Hydrocephalus and / or intraparenchymal cysts confirmed by cerebral ultrasound scan performed during the baby's in-patient stay;

15. Worst stage of retinopathy of prematurity in either eye at any time before discharge or death;

16. Length of stay in intensive, high dependency and special care (BAPM 2001: definitions in Appendix 4).

2.7 Data Collection Schedule

Information was collected at the following times:

- at trial entry – confirmation of eligibility and baseline data;
- daily until the post-natal age of 2 weeks - details of type of milk given and antibiotics administered;
- until discharge from hospital or death – data on suspected or proven episodes of NEC to facilitate classification, and microbiology data on samples taken from normally sterile sites;
- at 36 weeks post-menstrual age or sooner if discharged earlier, and at discharge – static data on clinical outcomes;
- 2 weeks post-natal and 36 weeks post-menstrual age – stool samples for detection of colonisation of the probiotic strain administered and other bacteria.

2.8 Sample Size & Power

Neonatal sepsis:
The percentage of babies with bloodstream infection in our pilot study was 44%. This included infection with skin commensals. The number of babies fulfilling criteria for the primary endpoint in this study will be lower, as infections with skin commensals

are excluded. Assuming a 5% level of statistical significance, a trial of 1,300 babies will have 90% power to detect a 40% relative risk reduction from 15% to 9.1%; likewise if the incidence is closer to 12%, a trial of this size will still have 90% power to be able to detect a 44% relative risk reduction from 12% to 6.7%, and a 44% reduction from 10% to 5.6%.

NEC:
The incidence of NEC is estimated to be 15%. This is based on NEC incidence at the Homerton Hospital over a 3 year period in babies less than 1,000g birthweight. A sample size of 1300 will have 90% power (at a 5% significance level) to be able to detect a 40% relative risk reduction in this outcome from 15% to 9%.

Death:
The incidence of death is also estimated to be 15%. This is based on survival of babies below 31 weeks gestational age in London extracted from pan-London data collected by the Thames Regional Perinatal Group. The sample size of 1300 will have 90% power (at a 5% significance level) to be able to detect a 40% relative risk reduction in this outcome from 15% to 9%.

2.9 Treatment Allocation

Allocation used a web-based randomisation service (with telephone back-up) based at the National Perinatal Epidemiology Unit (NPEU) Clinical Trials Unit, University of Oxford. The randomisation program used a minimisation algorithm to ensure balance on hospital, sex, gestational age at birth and whether or not randomisation occurs sooner than 24 hours after birth.

2.10 Interim Analyses and Stopping Rules

2.10.1 Interim analyses

Interim analyses were supplied, in strict confidence, to an independent Data Monitoring Committee (DMC) as frequently as its Chair requested and according to the DMC Charter as agreed by the DMC and TSC at their first combined meeting.

2.10.2 Criteria for determining termination of the trial

In the light of interim data and other evidence from relevant studies the DMC informed the Trial Steering Committee (TSC) that, in their view, there was no proof beyond reasonable doubt that the data indicated that the trial should be terminated. Recommendations to continue the trial were made to the TSC based, in part, on statistical considerations.

Appropriate proof beyond reasonable doubt cannot be specified precisely. A difference of at least 3 standard errors in the interim analysis of a major endpoint was needed to justify halting or modifying the study prematurely.

2.11 Independent Data Monitoring Committee Membership

Chair Professor Diana Elbourne
Statistician
Professor of Health Care Evaluation, The London School of Hygiene & Tropical
XXXX

Dr Benjamin Stenson
Consultant neonatologist, Simpson Centre for Reproductive Health
XXXX

Dr Jim Gray
Consultant Microbiologist, Birmingham Children's Hospital
XXXX

2.12 Trial Reporting

The trial will be reported according to the principles of the CONSORT statement[1].

3. DATA MANAGEMENT

3.1 Data Collection

All data for trial analyses, apart from stool samples, are routine clinical items that should be available from clinical notes or local microbiological laboratory records.

3.1.1 Form 1 – Trial Entry (1 form per baby)

At entry into PiPS, along with eligibility and randomisation data, maternal and obstetric information was abstracted from the maternal case notes and neonatal information was abstracted from the infant's medical records and entered onto Form 1 (Trial Entry Form). Form 1 was completed by the person randomising the infant into the PiPS trial.

3.1.2 Form 2 – Daily Data (1 form per baby per hospital)

Daily data collection until the post-natal age of 2 weeks collecting details of type of milk given and total daily volume; antibiotics and antifungals administered; and whether systemic ranitidine / proton pump inhibitor were given. Where a baby was transferred between hospitals within this 2 week time period, the form accompanied the baby.

3.1.3 Form 3 – Baby Transfer, Discharge or Death (multiple forms per baby)

When the baby was transferred to another hospital, discharged home or died, and at discharge home. Information about the infant's clinical outcomes whilst at that hospital were abstracted from the infant's medical records and recorded on Form 3. This form captured data from birth to discharge from that hospital or death at that hospital, on infections, whether full feeds were reached, results of any cerebral

ultrasound scans, treatment for patent ductus arteriosus, congenital malformations, retinopathy of prematurity, use of antimicrobials after the first 14 days since birth, any surgical procedures, use of level 1 and level 2 care, detail on any periods of omitting the intervention, and details of death or transfer to another hospital for continuing care. This form also captured data at 36 weeks post-menstrual age if the baby reached this age.

3.1.4 Form 4 – Abdominal Pathology (multiple forms per baby)

Data until transfer, discharge home or death around suspected or proven episodes of NEC whilst at that hospital to facilitate classification (definitions at Appendix 2).

3.1.5 Form 5 – Serious Adverse Event (SAE) & Suspected Unexpected Serious Adverse Reaction (SUSAR) (multiple forms per baby)

All details relating to a SAE or SUSAR including a description of the event, prescribed treatment details, concomitant treatments and further investigations.

3.1.6 Form 6 – Permanent Intervention Discontinuation or Trial Withdrawal (one form per baby)

Details regarding who and why the intervention was permanently stopped, and/or the baby withdrawn from the trial.

3.1.7 Stool Sample Analysis Report Form (two forms per baby)

This form was used to aid processing and recording of detailed colony types identified in faecal specimens collected as close as possible to 2 weeks post-natal age and 36 weeks post-mentrual age. Summary data were also recorded on these forms and entered onto the PiPS OpenClinica database. *B. breve* was identified using culture and Polymerase Chain Reaction (PCR) techniques at 2 weeks and culture only at 36 weeks. Quantities were recorded in units of log Colony Forming Units (CFU)/g for culture and ng DNA for PCR results. The following were identified using culture only: MRSA, VRE, MRGNB, ESBL.

3.1.8 Microbiology data (one record on database per baby)

Microbiological information obtained from the local microbiological laboratory on all samples taken from normally sterile sites, including blood, after 72 hours and before 46 weeks post-menstrual age, discharge home or death, whichever was soonest. Data recorded included details of organisms grown together with their antibiotic sensitivities. Summary data were entered directly onto the PiPS OpenClinica database, recording the number of blood cultures taken, episodes of infection with skin and non-skin commensals, what organisms were cultured and from which sterile sites, and if *B. breve* was cultured.

3.2 Data Entry, Cleaning and Validation

All completed Data Collection Forms (Forms 1 to 6) were sent to the NPEU CTU and double-entered onto a web-based clinical database, OpenClinica. Data were entered according to NPEU CTU OpenClinica data entry conventions. All personal details were entered into a Microsoft Access database. Validation programs performed a series of range, logic and missing data checks to identify any inconsistencies within and across forms on an ongoing basis. Some queries were resolved at NPEU according to predefined protocols, those that could be resolved were communicated to the appropriate centres by the Trial Co-ordinator and/or Data Manager, resolved where possible, and documented.

3.3 Database lock

The database will be locked for the final analysis on or close to 31st January 2014. However, information on deaths will continue to be collected after database lock and up until submission of the publication. The final lock of the database will therefore be the date of first submission of the publication.

3.4 Derivation of Variables

See Appendix 5 for derivation of variables.

3.5 Reliability

Data were double-entered into a MHRA compliant program by experienced data processors. Validity checks were run automatically by the computer program and 'unrealistic' values flagged, checked, and amended as necessary following NPEU CTU SOPs. On-site training of local staff by the trial research nurses was continuous throughout the trial.

Regular site visits were made by the study research nurse to ensure adherence to the protocol and to deal with any specific site issues. A major focus of these visits was to confirm that procedures to minimise the risk of cross contamination with the probiotic organism were followed both in the milk kitchen and in clinical areas.

Studies that have reported stool colonisation with the active probiotic bacterium, including our pilot, have reported cross-contamination of the placebo group. A system was established to monitor colonisation rates with the intention that the nurses reinforce training at any site where colonisation rates were outliers suggesting that there was excessive cross-colonisation.

All Form 4s containing data on Abdominal Pathology were reviewed by the Chief Investigator and one other clinician. The main purpose of the review was to confirm the occurrence, stage and number of any NEC episodes. These data were entered onto an Excel spread sheet and are considered the definitive data for the NEC related outcomes.

4. SERIOUS ADVERSE EVENT REPORTING

A Serious Adverse Event (SAE) is defined as the occurrence of an AE after trial entry where the death of the participant resulted or was otherwise threatened or where the participant required prolonged hospital stay; or resulted in persistent or significant disability or incapacity.

The group of infants in the PiPS trial have many serious adverse events and these were recorded in the case report forms. We anticipated the following SAEs in our infant population: death, culture positive infection with organisms other than *Bifidobacterium breve* strain BBG, necrotising enterocolitis or focal intestinal perforation, broncho-pulmonary dysplasia, intracranial abnormality (haemorrhage or focal white matter damage) on cranial ultrasound scan or other imaging, pulmonary haemorrhage, patent ductus arteriosus, retinopathy of prematurity requiring retinal surgery. All of these conditions were recorded on the case report form but none required immediate reporting to the sponsor.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as the occurrence of an adverse reaction after trial entry the nature or severity of which is not consistent with the known safety profile of the study intervention.
The only recognised possible adverse reactions associated with probiotic administration are:

- Positive culture of the probiotic organism *Bifidobacterium breve* strain BBG from a normally sterile site – This is a very rare event and with this organism it has only been reported once.
- Intestinal obstruction caused by starch – this was reported when this product was first used but has not been reported with the product prepared as it will be in this study administering only the supernatant after suspension and allowing the starch to settle.

Planned reporting procedures of the PIs and CI

- Any event which is described in the Protocol as expected will not be reported to the sponsor in an expedited manner.
- The CI will ensure that PIs are asked about any untoward SAEs / SUSARs occurring since the previous contact.
- The PIs will ensure that all anticipated SAEs are recorded in the infant's case report form; these were reviewed by the DMC during the trial.
- The PIs will report SUSARs to the Clinical Trials Unit, NPEU by telephone/fax/email at the earliest opportunity and within one working day of them becoming aware of the occurrence.
- The PI will provide a written detailed report to the Clinical Trials Unit, NPEU within 3 days of first knowledge of a fatal SUSAR and within 7 days of a non-fatal SUSAR.

- The CI or person with delegated responsibility will review all SAEs/SUSARs as reported on Form 5 within one working day of their receipt, and will consider causality and expectedness. Where there is any suspicion that an event is linked to the PiPS trial intervention that event will be classified as a SUSAR and reported accordingly.
- All expected SAEs will be recorded on the case report form, and reviewed by the Data Monitoring Committee during the trial. SUSARs are to be sent to the chair of the DMC for regular review.
- The CI will notify the MHRA and South Central - Oxford A REC and Sponsor of all SUSARs reported to them by the PIs within 7 days if the SUSAR is linked to a death or considered life-threatening; with additional information sent within a further 8 days. All other SUSARs will be notified to the MHRA/REC within 15 days of first knowledge of the event.
- As soon as practicable, the CI will inform PIs in all participating Neonatal Intensive Care Units in PiPS of the notified SUSAR.
- The CI will maintain a detailed record of all SAEs and SUSARs reported to them by the PIs. The record will be kept electronically in a secure computer file at the NPEU, with off-site back up. The CI will send details of this record in the annual safety report to the MHRA and South Central - Oxford A REC and Sponsor. A copy will be made available to the MHRA at any time on receipt of a written request.
- SUSARs will be reported for each infant for the period of the trial supplementation **plus** two weeks or discharge from hospital (whichever is first).

5. PROTOCOL VIOLATIONS AND DEVIATIONS

5.1 Protocol Violation

A protocol violation was defined as failure to comply with the final study protocol as approved by Ethics Committee and Research Department. A violation is a serious non-compliance with the protocol resulting from error, fraud or misconduct and results in the exclusion of a patient from the study. A violation will be reported to the Sponsor and Ethics Committee as soon as possible.

5.2 Protocol Deviation

A protocol deviation is a less serious non-compliance, for instance:

• Inclusion/ exclusion criteria not fulfilled
• Incorrectly performed/ missing tests

6. PATIENT GROUPS FOR ANALYSIS

6.1 Post-randomisation exclusions

Losses to the trial post randomisation are defined as any of the following:-

- babies for whom a valid consent was not received;
- babies for whom consent to use their data was withdrawn.

The numbers (with percentages of the randomised population) of post-randomisation exclusions will be reported by randomised treatment group, and reasons summarised.

Parents can specify whether data collected up to the point of withdrawal can be used. If the response is 'No', then they will be considered post-randomisation exclusions. If the response is 'Yes', then they will be reported as 'missing' for any data not collected after withdrawal.

6.2 Primary Analysis Strategy

For the primary analysis, infants will be analysed in the groups into which they were randomly allocated e.g. comparing the outcome of all infants allocated *B. breve* with all those allocated placebo regardless of intervention received.

6.2.1 Descriptive analysis population

Baseline demographic and clinical characteristics – all infants randomised for whom we have data available, excluding any post-randomisation exclusions (see section 6.1).

6.2.2 Comparative analysis population

All infants will be included in the analysis except any post-randomisation exclusions (see section 6.1).

6.2.3 Safety analysis population

All infants will be included in the analysis except any post-randomisation exclusions (see section 6.1).

6.2.4 Interim analysis population

The interim analyses presented baseline data, and primary and secondary outcomes. Some outcomes are based on microbiology data retrieved from electronic laboratory sources. These data are requested in batches for babies who are known to have completed the study (i.e. forms received indicating that the baby had died or been discharged home). In addition, if a baby was still in hospital at the time of analysis, they were still at risk of NEC or death. It was therefore considered appropriate to present all outcome data on the same group of babies described as 'completers' (i.e. babies died or discharged home).

7. DESCRIPTIVE ANALYSES

7.1 Representativeness of Trial Population and Participant Throughput

We will summarise the flow of participants through each stage. Specifically, for each treatment group we will report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and analysed for the primary outcome. Protocol deviations from the study will be described, together with reasons.

The number of ineligible patients randomised, if any, will be reported, with reasons for ineligibility.

The total number of eligible babies was not collected during the conduct of this study as it was considered heavy on resources and would not be sufficiently reliable. It is planned to use data collected routinely by the Neonatal Data Analysis Unit (NDAU) to assess representativeness of the trial population for the recruiting hospitals and the broader population of English neonatal admissions during the recruitment period of the PiPS trial.

7.2 Baseline Comparability of Randomised Groups

Baseline characteristics of each treatment group will be described (all data taken from Form 1). See Appendix 6, Tables 1.1 and 1.2 for characteristics included.

Numbers (with percentages) for binary and categorical variables and means (and standard deviations), or medians (with lower and upper quartiles) for continuous variables will be presented.

7.3 Analysis of Adherence

Adherence to intervention will be assessed by calculating the total days between post-menstrual age at first dose and last dose and subtracting the total number of days when the intervention was stopped temporarily. Since this duration will depend on the babies' gestational ages at birth, (i.e. the 'time at risk' will be different depending on the gestation of the baby at birth), this will be expressed as a proportion of the total days that a baby should have been on the intervention (i.e. from the date of randomisation to the date at which the baby was 36 weeks post-menstrual age). These data will be presented by gestational age at birth using categories as used in the minimisation algorithm, in order to assess patterns of adherence for different gestational ages.

Adherence to study protocol will be assessed using post-natal age in hours at randomisation and gestational age at birth.

These data will be presented as means and standard deviations, if approximately normally distributed, by treatment group, and compared using differences in means and 95% confidence intervals (CI). If the data are considered non-normal then medians and interquartile ranges will be presented with comparisons made using differences in medians and 95% CIs.

7.4 Unblinding of Randomised Treatments

Numbers and percentages of any unblinding of treatments will be reported.

8. COMPARATIVE ANALYSES

8.1 Analysis strategy

An adjusted analysis will be performed on all comparative analyses adjusting for the variables used in the minimisation algorithm - hospital, sex, gestational age at birth (23w, 24w, 25w, 26-27w and 28-30w) and whether or not randomisation occurs sooner than 24 hours after birth[2] (see section 2.9) The adjusted analysis will also account for the correlation of outcomes among babies from multiple births included in the trial.

Risk ratios will be estimated using generalised estimating equations (GEE), or a similar method. This method of analysis will account for the correlation in outcomes between multiple births. Binary outcomes will be analysed using log binomial regression models and results will be presented as adjusted risk ratios with corresponding confidence intervals (CI). If the model does not converge, then centre will be removed as a stratification factor in the first instance. If the model is still unstable then log Poisson regression models with robust variance estimation will be used[3]. Continuous outcomes will be analysed using linear regression models and results will be presented as adjusted differences in means with associated confidence intervals. Transformations will be applied for non-normal data.

Outcomes will be summarised with counts (percentages) for categorical variables; the mean (standard deviation [SD]) for normally distributed continuous variables, or the median (interquartile [IQR] or entire range, whichever appropriate) for other continuous variables.

To establish the magnitude and direction of the treatment effects, comparative statistical analysis will entail calculating the adjusted risk ratios (RR) plus confidence intervals (CI) for binary outcomes, the adjusted mean differences (CIs) for normally distributed continuous outcomes, or the unadjusted median differences (plus CIs) for skewed continuous variables (unless the data can be transformed to Normality).

95% Confidence Intervals (CIs) will be presented to compare the risks of the primary outcomes between the treated and placebo groups. 99% CIs will be presented for all other outcomes.

Identification of *B. breve* BBG from stool samples at 2 weeks is made using both culture and PCR techniques. The analysis using colonisation status of *B. breve* BBG based on stools collected at 2 weeks will report that *B. breve* BBG is positive if either technique reports a positive result. The 36 week data will be based on culture results only.

8.2 Analyses of Primary Outcomes

8.2.1 Primary analysis

The primary analysis of the three primary outcomes will be under intention-to-treat i.e. according to the randomised groups for whom we have an outcome, and excluding post-randomisation exclusions (see section 6.1), and will adopt the analysis strategy set out in 8.1.

8.2.2 Secondary analyses

A secondary analysis of all three primary outcomes will be performed according to the colonisation status of the baby at 2 weeks post randomisation. This analysis will be conducted on the analysis population as defined in section 6.2.2 for babies for whom colonisation data are available, and will adopt the analysis strategy set out in 8.1. Data will be presented by whether or not the baby was colonised with *B.breve* BBG.

8.2.3 Pre-specified Subgroup Analysis

A statistical test for interaction will be used to assess the consistency of the adjusted treatment effect on the primary outcomes. The following pre-specified subgroup analyses will be performed on the primary outcomes stratified by:

- whether randomised in the 1st or 2nd 24 hours after birth
- gestational age at birth as per minimisation: 23w, 24w, 25w, 26/27w, 28/29/30w.
- male versus female
- colonised versus not colonised at 2 weeks
- gestational age <28+0 versus ≥28+0

Results will be presented on forest plots with the interaction results alongside.

The subgroup analysis by age at randomisation is included as an unbiased surrogate marker for age at first dose. The additional subgroup analysis by gestational age is included in order that a comparison with the ProPrems study[4] can be made. The subgroup analysis by colonisation status is included as an unbiased assessment of the effect of colonisation status taking into account the randomised groupings. This will be used to complement the secondary analysis described in section 8.2.2.

8.2.4 Exploratory Analysis

For those babies who are colonised with *B.-breve* BBG at 2 weeks an exploratory analysis will be undertaken to investigate if adjusting for the quantity of *B. breve* BBG found using culture and separately using the PCR technique impacts on the adjusted effect estimates of the primary outcomes. The adjusted analysis described in section 8 will be extended to include the quantitative results of the culture and PCR techniques. Results will be considered as hypothesis generating only.

The adjusted model will be altered to include gestational age as a continuous variable, rather than as a categorical variable, to evaluate the impact of this on the effect estimate.

8.3 Analyses of Secondary Outcomes

8.3.1 Primary analysis

The primary analysis of all secondary outcomes will be under intention-to-treat i.e. according to the randomised groups for whom we have an outcome, and excluding post-randomisation exclusions (see section 6.1), and will adopt the analysis strategy set out in 8.1.

For specific secondary outcomes the following analyses will be undertaken:The number of babies with isolates of organisms other than skin commensals from a normally sterile site other than blood (secondary outcome 6) will be summarised by trial arm, by type of sterile site as well as overall. A relative risk and 99% confidence interval will be presented for the number overall only.

Stool flora (secondary outcome 10) data are based on culture results only and will be analysed for each of the type of flora cultured (MRSA, VRE, ESBL) at 2 weeks post-natal and at 36 weeks postmenstrual age, with relative risks and 99% confidence intervals for each.

Age at achieving full enteral nutrition (secondary outcome 11) will be analysed as a time to event outcome, with the time defined as post-natal age and the event as achieving full enteral nutrition (defined as 150 ml/kg/day for 1 day). The analysis will use Cox-proportional hazards methods adjusting for the minimisation factors as set out in section 8.1. A hazards ratio with 99% confidence intervals for the treatment group comparison will be presented.

Change of weight z-score (secondary outcome 12) will be assessed using an Analysis of Covariance (ANCOVA) to adjust for weight at birth.

Broncho-pulmonary dysplasia (BPD) (secondary outcome 13) will be considered positive if categorised as moderate or severe according to the definitions detailed in Appendix 3.

Worst stage of retinopathy (secondary outcome 15) will be categorised as grade 3 or

above and compared to grade 2 or less for the purposes of analysis.

8.3.2 Secondary Analysis

A secondary analysis of all secondary outcomes will be performed according to the colonisation status of the baby at 2 weeks post randomisation. This analysis will be conducted on the analysis population as defined in section 6.2.2 for babies for whom colonisation data are available , and will adopt the analysis strategy set out in 8.1. Data will be presented by whether or not the baby was colonised with *B. breve* BBG.

8.4 Significance Levels and Multiplicity

For all analyses on the primary outcomes 95% confidence intervals (CI) will be presented, and a significance level of 5% (consistent with a 95% CI) will be used to indicate statistical significance.

For all analyses on secondary outcomes 99% confidence intervals (CI) will be presented, and a significance level of 1% (consistent with a 99% CI) will be used to indicate statistical significance.

p-values will not be presented for comparative analyses but will be presented for tests of interaction.

8.5 Missing Data

All comparative analyses will be carried out ignoring missing data. The reason for missing data (consent withdrawn, lost to follow-up, removed from study due to serious side effects, death) will be indicated where possible. The primary analysis on each of the primary outcomes will be repeated using multiple imputation techniques if more than 5% of each of the primary outcomes is missing and the missing completely at random assumption is considered appropriate. These will be treated as a sensitivity analysis. For missing colonisation data, generalisability will be assessed using cross tabulations of baseline characteristics for babies with missing colonisation data versus babies with valid colonisation data.

8.6 Statistical Software Employed

Stata statistical analysis software will be used for all analyses.

9. SAFETY DATA ANALYSIS

9.1 Serious Adverse Events

Any serious adverse event occurring whilst an infant was in the PiPS trial, up to death or discharge home or the date of the final database lock, will be recorded and tabulated in full. A comparison of serious adverse events between each arm of the trial will be assessed.

10. ADDITIONAL EXPLORATORY ANALYSIS

The following further exploratory analyses will be performed to provide context to the results or to generate hypotheses for future testing:

1) A logistic regression analysis to study determinants of successful colonisation with *B. breve* BBG at 2 weeks in those babies allocated to receive probiotic. A forward stepwise regression model will be used to assess the following factors:

- postnatal age at receiving first dose of probiotic
- duration of antibiotic use in the first 14 days
- type of antibiotics received
- type of milk received
- postnatal age at starting milk
- number of days any milk received in first 14 days
- gestational age at birth
- singleton/multiple.

2) An adjusted analysis (according to the strategy set out in section 8.1) to investigate the treatment effect on the use of post-natal corticosteroids given to prevent BPD.

3) Cross-tabulations of

- colonisation with *B. breve* BBG at 2 weeks postnatal age by colonisation with *B. breve* BBG at 36 weeks postmenstrual age by randomisation group;
- post-natal age at randomisation by randomisation group;
- post-natal age at first dose by randomisation group;
- post-natal steroid use by randomisation group.

4) A summary by treatment group of the following variables:-

- number of episodes of NEC ≥ stage 2
- worst stage of NEC

- age of onset of NEC ≥ stage 2
- surgical NEC
- fatal NEC
- Spontaneous Intestinal Perforation

5) In order to be able to give the outcome used in the meta-analyses we will report the outcome of number of babies with any positive blood culture (i.e. the primary outcome and secondary outcome 2 combined) by treatment group.

Any analyses not specified in this analysis plan will be exploratory in nature and a 1% significance level will be used to declare statistical significance; 99% confidence intervals will be presented.

11. DEVIATION FROM ANALYSIS DESCRIBED IN PROTOCOL

None yet.

12. REFERENCES

12.1 NPEU Clinical Trials Unit Standard Operating Procedures

ST 104 Interim Statistical Analysis
ST 105 Statistical Analysis Plan
ST 106 Final Statistical Analysis and Reporting

12.2 Trial documents

NPEU OpenClinica Data Entry Guide version 1

All other trial documents are available at: https://www.npeu.ox.ac.uk/pips

12.3 References

1) Schulz KF, Altman DG, Moher D, Group for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *British Medical Journal* 2010; 340: 698-702.
2) Brennan C. Kahan and Tim P. Morris. Analysis of multicentre trials with continuous outcomes: when and how should we account for centre effects? *Statistics in Medicine* 2013; 32: 1136-1149
3) Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol.* 2004 Apr 1;159(7):702-6.
4) Susan E. Jacobs, Jacinta M. Tobin, Gillian F. Opie, Susan Donath, Sepehr N. Tabrizi, Marie Pirotta, Colin J. Morley and Suzanne M. Garland. Probiotic Effects on Late-onset Sepsis in Very Preterm Infants: A Randomized Controlled Trial. *Pediatrics* 2013; 132:1055-1062

12.4 Acknowledgements

This Statistical Analysis Plan is based on a template written by Ed Juszczak and Ros Weatherall whilst at the Centre of Statistics in Medicine, University of Oxford.

13. APPENDICES

Appendix 1: Rationale and definitions for microbiological endpoints

The primary outcome: *'An episode of blood stream infection, with any organism other than a skin commensal, diagnosed on a sample of blood drawn after 72 hours and before 46 weeks post-menstrual age, death or discharge from hospital whichever is soonest. Skin commensals include coagulase negative staphylococci (CoNS) and Corynebacteria.'*

Late onset blood stream infection in the preterm baby carries high mortality and morbidity; this is particularly true for infections with *Staphylococcus aureus* and Gram negative bacilli (GNB) which make up around 15% of positive blood cultures. The main reservoir of these organisms in the baby is in the gut from which it is believed that they invade the bloodstream by translocation of the intestinal wall. If probiotic administration is to be effective in reducing infection in the newborn it is most likely that it will be through a combination of reducing colonisation of the gut by these organisms and promoting intestinal epithelial health.

The majority of positive blood cultures are with Coagulase-negative Staphylococci (CoNS). These organisms likewise colonise the gut of infants but they are also important skin commensals of healthcare workers; this reservoir of colonisation will not be affected by probiotic administration to infants. CoNS bloodstream infection is thought usually to arise as a result of colonisation of an intravascular device, most importantly an intravenous central feeding line, during handling and manipulation by healthcare workers, rather than from bacterial translocation through the intestinal wall. Thus while probiotic use, if it is associated with better nutrition and better general health, might reduce need for intravascular devices and might be related to less CoNS sepsis it seems probable that the greater benefit of probiotics in neonatal infection will be through reduction of the more serious infections with organisms that have colonised the intestine such as GNB, *S. aureus* and fungi such as Candida.

Furthermore there is a difficulty in accurate diagnosis of CoNS infection. While a positive blood culture with the clearly pathogenic *Staphylococcus aureus* or GNB is taken as definite evidence of infection it is widely acknowledged that many CoNS positive blood cultures are contaminants, arising largely through deficient blood culture technique with inadequate skin cleansing. Many schemes have been presented, to explore whether the presence of an organism in a culture sample represents real infection rather than a contaminant. These involve different combinations of clinical signs (lethargy, temperature instability, etc.) and laboratory markers of sepsis (WBC counts, CRP etc.). None of these is accepted as a gold standard and if used in a clinical trial such as PiPS would result in a significant increase in the burden of data collection for participating centres without clear evidence of benefit in the specific circumstances of this randomised trial.

In summary: because of the greater clinical importance of blood stream infection with non skin commensals, the possibility that they are more likely to be reduced by probiotic administration and in the cause of simple clearly defined items for data collection it has been agreed that the microbiological primary endpoint for this study should be blood stream infection with non skin commensals, i.e. positive cultures with bacteria such as *E. coli, Klebsiella, S. aureus* and with fungi such as *Candida*.

Secondary outcomes:

While the single most important microbiological clinical outcome is reduction of blood stream infection with non skin commensals there are other possible effects of probiotic use that are important to study:

Infection with skin commensals, secondary outcomes #2-4:
Because details of clinical events and markers of sepsis are not being collected around episodes of suspected infection, the total number of positive blood cultures with skin commensals (the majority of which will be CoNs) will include contaminants; it will however give a guide as to whether or not probiotic use is impacting on skin commensal sepsis as the contaminants should be balanced between the two arms of the study. This information will be augmented by studying whether or not there is a difference in the extent of sampling (secondary outcomes 3&4) in the two arms.
Infections with pathogens: GNB, S. aureus etc. by organism and antibiotic resistance, secondary outcome #5:
The bowel provides a major reservoir for antibiotic resistant bacteria and is also an important site for the transfer of antibiotic resistance genes. If probiotics are not associated with the hoped for reduction in serious blood stream infection they may nonetheless impact upon the type of organisms causing infection and be associated with less antibiotic resistance. To explore this, the types of organisms causing blood stream infection and their patterns of antibiotic resistance will be studied in the two arms of the study.
Blood culture negative episodes of infection:
A further complication in the accurate assessment of the burden of infection is the difficulty of reliably identifying clinical episodes that are considered by the attending staff to be infections but are associated with a negative blood culture; this may arise because the sample of blood is too small but is more often because the baby, at the time of sampling, is already on antibiotics which inhibit bacterial growth. The total number of samples taken, secondary outcome #4, will to some extent provide a surrogate for this.
Data collection to support these endpoints:
Investigators will provide the study centre with details of admission and discharge dates; this might involve multiple hospitals per baby. Microbiological data will be obtained directly from hospital microbiological laboratories who will be asked to provide a download with details of all microbiological investigations from admission, including time and site of sampling and details of any positive cultures with information about antibiotic resistance.
The total days of antibiotic use, for treatment of suspected or proven sepsis, and excluding prophylactic use, will be collected using the study data collection forms.
Stool samples will be collected for the study as close as possible to 2 weeks post-natal age and 36 weeks postmenstrual age and sent to the study centre where they will be examined for colonisation with *Bifidobacterium breve* BBG and subjected to quantitative microbiology to study patterns of microbiological colonisation and antibiotic resistance.

Appendix 2: Definitions of Necrotising Enterocolitis

NEC will be classified using Modified Bell's criteria[1] with further minor modification excluding recording of positive occult blood in stools and noting of bowel sounds:

Bell stage	Systemic signs	Gastro-intestinal signs	Radiographic signs
Stage IIA (Definite NEC: mildly ill)	Increased desaturations and/or bradycardia Temperature instability Lethargy	Increased pre-feed gastric aspirate Definite abdominal distension Possible abdominal tenderness Possibly bloody stools	Definite abdominal dilatation Pneumotosis intestinalis
Stage IIB (Definite NEC: moderately ill)	As Stage IIA with platelets <100 x 10_{12} and/or metabolic acidosis: base excess <-8meq/l	Abdominal distension with definite tenderness Possible abdominal wall oedema and/or erythema	As IIA with portal vein gas Possible ascites
Stage IIIA (Advanced NEC: bowel intact)	As IIB plus mixed acidosis: pH <7.2 DIC Neutropaenia <1x10_9/l Severe apnoea Hypotension requiring inotropes	Generalised peritonitis with severe tenderness with abdominal wall induration	As IIA with definite ascites
Stage IIIB (Advanced NEC: bowel perforated)	As IIIA	As IIIB	As IIIA with pneumoperitoneum

References

1. Walsh MC, Kliegman RM. Necrotising enterocolitis: treatment based on staging criteria. Pediat Clin North Am, 1986;33:179-201

Appendix 3: Bronchopulmonary dysplasia (BPD)

Principal definition for secondary outcome: A baby who is still receiving supplementary oxygen at 36 weeks postmenstrual age.

BPD is one of the most important complications of preterm birth, lengthening hospital stay, increasing the burden on parents particularly through the frequent need for home oxygen and being associated with longer term morbidity. The definition above, which is the standard version that has been used in many clinical trials and clinical studies of neonatal outcomes is imprecise in that whether or not a baby receives oxygen is to a considerable extent dependent upon local practice and the whim of the clinical staff looking after the baby on that particular day. There has been considerable interest in making the definition more objective either by relating it more precisely to physiological measures of gas exchange[1,2] or by strengthening the underpinning clinical information[3]. This outcome is of particular importance and interest for this study since in the pilot study undertaken by the investigators there was a significant reduction of BPD in association with *B. breve* BBG colonisation. The 'physiological' assessment of BPD severity is not yet adequately evaluated in terms of its reliability and reproducibility to use it as an outcome measure in a clinical trial such as this. Clinical data to support the categorisation of babies using the system proposed by the NICHD[3] has been collected for babies in EPICure 2 (all births <27w in 2006). Preliminary analysis of data from 869 of 870 possible surviving infants show a significant relationship between the severity of BPD using this classification at 36w pma and the likelihood of going home in oxygen. It is proposed that babies in PiPS are likewise classified at 37w using this scheme and the numbers of babies with different degrees of severity of BPD compared between the probiotic and placebo groups:
No BPD: in air by 28d post natal age.
Mild BPD: in oxygen at 28d pna but in air and not receiving mechanical ventilatory support at 36w post menstrual age.
Moderate BPD: in oxygen but <30% or≤0.1l/min or on mechanical support in air at 36w post menstrual age.
Severe BPD in oxygen ≥30% or >0.1l/min at 36w post menstrual age

References

1. Walsh MC, Wilson-Costello D, Zadell A, Newman N, Fanaroff A. Safety, reliability and validity of a physiological definition of broncho-pulmonary dysplasia. J Perinatol. 2009;23:451-456
2. Quine D, Wong CM, Boyle EM, Jones JG, Stenson BJ. Non-invasive measurement of reduced ventilation:perfusion ratioand shunt in infants with bronchopulmonary dysplasia: a physiological definition of the disease. Arch Dis Childh. 2006;91:F409 –F414
3. Jobe AH & Bancalari E. NICHD/NHLBI/ORD Workshop Summary: Bronchopulmonary Dysplasia. Am J Respir Crit Care Med. 2001;163:1723-1729

Appendix 4: BAPM definitions of intensive, high-dependency and special care

Standards for hospitals providing intensive and high-dependency care: British Association of Perinatal Medicine, 2001

Intensive Care

Any baby who is:

1. receiving any respiratory support via a tracheal tube and in the first 24 hours after its withdrawal
2. receiving NCPAP for any part of the day and less than five days old
3. below 1000g current weight and receiving NCPAP for any part of the day and for 24 hours after withdrawal
4. less than 29 weeks gestational age and less than 48 hours old
5. requiring major emergency surgery, for the pre-operative period and post-operatively for 48 hours
6. requiring complex clinical procedures:

- full exchange transfusion
- peritoneal dialysis
- infusion of an inotrope, pulmonary vasodilator or prostaglandin and for 24 hours afterwards

7. a baby on the day of death.

High Dependency Care
Any baby who is:
1. receiving NCPAP for any part of the day and not fulfilling any of the criteria for intensive care
2. below 1000g current weight and not fulfilling any of the criteria for intensive care
3. receiving parenteral nutrition
4. having convulsions
5. receiving oxygen therapy and below 1500g current weight
6. requiring treatment for neonatal abstinence syndrome
7. requiring specified procedures that do not fulfil any criteria for intensive care:

- Care of an intra-arterial catheter or chest drain
- Partial exchange transfusion
- Tracheostomy care until supervised by a parent

8. requiring frequent stimulation for severe apnoea.

Special Care
Special care is provided for all other babies who could not reasonably be expected to be looked after at home by their mother.

Appendix 5 – Derivation of variables

	Derived Variable	Derivation	Contributing questions	Form: Q no
1	Gestational age	The number of weeks between the expected delivery date and the date of birth subtracted from 40 weeks. The gestational age will be displayed to 1 decimal place.	Baby's date of birth Expected delivery date	F1:A2 F1:A1
2	Birth weight z-score	Calculated according to Pan H, Cole TJ. LMSgrowth, a Microsoft Excel add-in to access growth references based on the LMS method. Version 2.77. http://www.healthforallchildren.co.uk/; 2012. The British 1990 reference chart is referenced. Cole TJ, Freeman JV, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. *Statistics in Medicine* 1998;17:407-429.	Expected delivery date Date of birth Sex Weight at birth	F1:A1 F1:A2 F1:A3 F1:D7
3	CRIB II	Calculated according to Parry et al. Lancet 2003; 361: 1789-91.	Birth weight Gestational age Sex Temperature at admission Base excess	F1:D7 Derived 1. F1:A3 F1:D10 F1.D12
4	Day of first feed	On form 2, the first day from DOB when the 'No Milk given' box is not ticked will be selected as the day of first feed.	From form 2; No Milk	F2:C
5	Any Formula Milk in first 2 weeks	The type of feed will be categorised into 'maternal breast milk' yes / no (preterm formula or term formula). This variable will be yes in any formula was received in the first 2 weeks, otherwise it will be no.	From form 2; Preterm formula Term Formula	F2:C F2:C
6	Maternal breast milk in first 2 weeks	The type of feed will be categorised into 'maternal breast milk' yes / no (expressed maternal milk or fed directly from the breast), This variable will be yes in any breast milk was received in the first 2 weeks, otherwise it will be no.	From form 2; Expressed maternal milk Fed directly from breast	F2:C F2:C
7	Days on antibiotics (post 72 hours)	For day 4 to 14, each day one or more of the antibiotics listed in form two were taken is counted as one day.	From form 3; For how many days in total were antibiotics taken?	F3:B8 F2:D

	Derived Variable	Derivation	Contributing questions	Form: Q no
		The response to question 'For how many days in total were antibiotics taken?' will be summed across all form 3s to give the number of days antibiotics were taken after 14 days. Therefore 'Days on antibiotics' will be the sum of days 4 to 14 and the total days after day 14.	From form 2; Any penicillin Any aminoglycoside Any cephalosporin Any glycopeptide Any carbapenem Any β lactum / inhibitor Other antiobiotic	
8	Days on antifungals to treat suspected or proven infection. (post 72 hours)	For the day 4 to 14, each day antifungal to treat suspected or proven infection was taken, as collected in form two will be summed to give days taken from day 4 to 14. The response to question 'For how many days in total were antifungals taken?' will be summed across all form 3s to give the number of days antifungals were taken after 14 days. Therefore 'Days on antifungals to treat suspected infection' will be the sum of days 1 to 14 and the total days after day 14.	From form 3; For how many days in total were antifungals taken? From Form 2; Antifungal to treat suspected or proven infection.	F3:B8 F2:D
9	Time to first full feed	The time from randomisation to first full feed will be derived from the number of days between the randomisation date and the date of first full feed. If multiple dates are given, date will initially be queried, if not resolved the first date given will be used.	While in this hospital did the baby reach full feeds for the first time? Date of first full feed.	F3:B3
10	BPD, none, mild, moderate and severe	None and Mild; cannot be defined with data collected, no information available at 28 days post natal age . Moderate; in oxygen <30% or ≤0.1l/m or on mechanical support at 36 weeks post menstrual age. Severe; in oxygen ≥30% or >0.1l/m at 36 weeks post menstrual age.	Was the baby still receiving mechanical respiratory support? Was the baby receiving supplementary oxygen? Oxygen <30% or ≥ 30% Oxygen ≤0.1l/m or >0.1l/m	F3:C2i F3:C2iii
11	Hydrocephalus	If the baby does not have valid cerebral	While in this hospital	F3:B4

	Derived Variable	Derivation	Contributing questions	Form: Q no
	and / or intraparenchymal cysts	ultra sound scan data (i.e. While in this hospital did the baby have any cerebral ultrasound scans? is blank) then this variable will be missing. If hydrocephalus, porencephalic cyst or periventricular leucomalacia was present on either the left or right side, at any hospital, this variable will be yes. Any other non-missing ultrasound result will make this variable no.	did the baby have any cerebral ultrasound scans?	
12	Total hospital stay	The last date in hospital will be the latest of the discharge home date and the death date. Total hospital stay will be last date in hospital minus the baby's date of birth plus one.	Discharged home Death Date of birth	F3:D1 F3:D3 F1:A2
13	Intensive care stay	The sum of all intensive care days at each hospital.	While in this hospital, what was the total number of days in intensive care?	F3:B10
14	High dependency care stay	The sum of all high dependency care days at each hospital.	While in this hospital, what was the total number of days in high dependency care?	F3:B10
15	Special care stay	Total stay minus the intensive care days and the high dependency care days.	Discharged home Death Date of birth While in this hospital, what was the total number of days in intensive care / high dependency care?	F3:D1 F3:D3
16	Post menstrual age at first dose. (hours)	The number of weeks between the expected delivery date and the date of first dose from 40 weeks.	First dose date Expected delivery date	F1:A2 F1:A1
17	Post menstrual age at last dose. (days)	Date of last dose is the maximum of permanent discontinuation date, last date trial intervention was given and withdrawal date. The number of weeks between the expected delivery date and the date of last	Permanent discontinuation date Study Withdrawal date Last date trial intervention given. Expected delivery	F3:C5 F3:B12 F6:B1 F1:A1

	Derived Variable	Derivation	Contributing questions	Form: Q no
		dose subtracted from 40 weeks.	date	
18	Total duration.	Date of last dose is the maximum of permanent discontinuation date, last date trial intervention was given and withdrawal date. Total duration is the difference between the first dose date and last dose date plus one. If the days total days of interruption is greater than the total duration this will be queried. If not resolved both the days of interruption and the total duration will take missing values.	Permanent discontinuation date Study Withdrawal date Last date trial intervention was given. Date of first dose.	F3:C5 F3:B12 F6:B1 F1:B3
19	Total duration of temporary interruptions.	The sum of all temporary discontinuations across all hospitals. If the days total days of interruption is greater than the total duration this will be queried. If not resolved both the days of interruption and the total duration will take missing values.	For how many days in total was the trial intervention discontinued.	F3:B12
20	Percent of recommended doses taken.	The date at which the baby will be 36 weeks post menstrual will be the expected delivery date minus 28 days (4 weeks). The number of days between the randomisation date and the date the baby reaches 36 weeks post menstrual age gives the number of recommended days which is the denominator for the percentage. The number of days the intervention is taken is the total duration minus the total days of interruption. The percent of recommended doses taken is the number of days the intervention was taken, divided by the recommended days. The result is multiplied by 100 to give a percentage. If the days total days of interruption is greater than the total duration this will be	Expected delivery date Randomisation date. Total duration Date of first dose. For how many days in total was the trial intervention discontinued.	F1:A1 derived19 F1:B3 F3:B12

	Derived Variable	Derivation	Contributing questions	Form: Q no
		queried. If not resolved both the days of interruption and the total duration will take missing values.		
21	Post natal age at randomisation (hours).	The number of hours between randomisation date and time and the date of birth, date and time.	Date of birth Time of birth Date of first dose Time of first dose	F1:A2
22	Post natal age at first dose (hours).	The number of hours between the first dose date and time and the date of birth, date and time.	Date of birth Time of birth Date of last dose Time of last dose	F1:A2 F1:B3
23	Multiples	Identification of siblings; Any multiple babies born on the same day to a mother with the same DOB will be included in one cluster. Singletons will be in a cluster of size 1. Checks will ensure that a multiple baby will not be included in a cluster larger than the number of babies born.	Baby's DOB Mother's DOB Multiple/Singleton Number of babies born	F1:A2 F1:C3 F1:A4 F1:D8

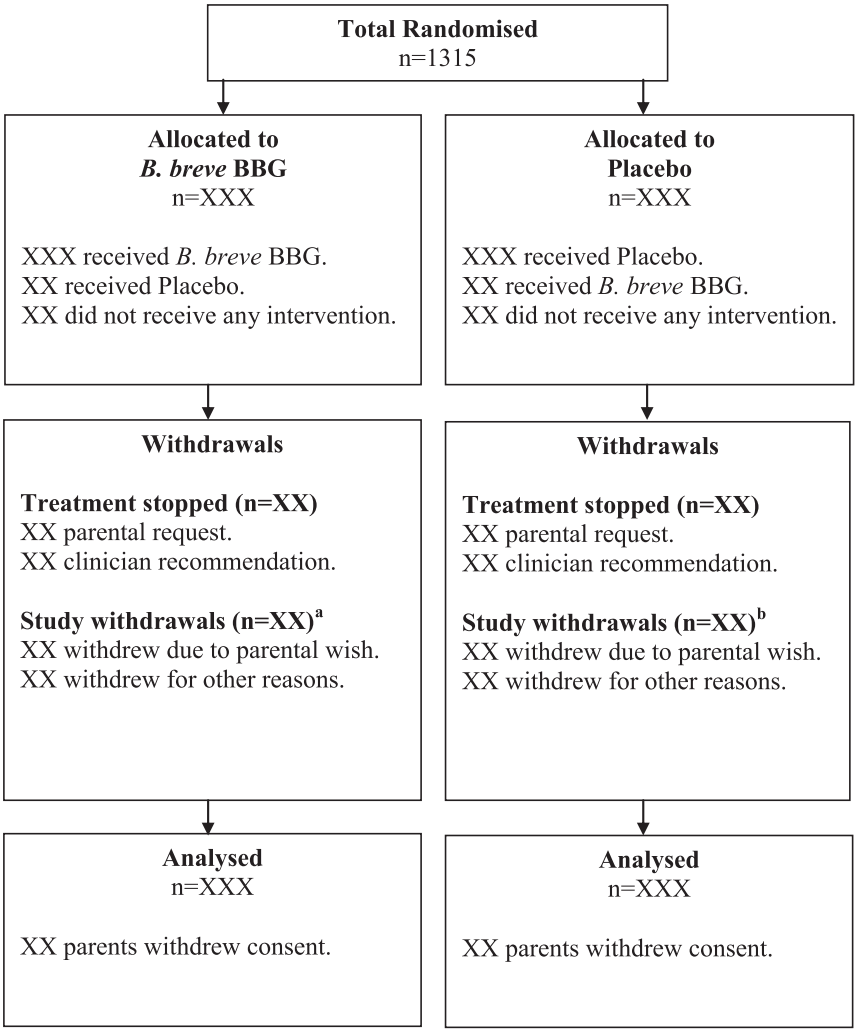
Appendix 6 – Dummy Tables

Outline of tables

		ALL PARTICIPANTS	ITT POPULATION	
			BY TRIAL ARM	BY COLONISATION
FIGURE 1	PARTICIPANT FLOW	X		
TABLE 1.1	BASELINE DATA: MOTHER'S CHARACETERISTICS		X	X
TABLE 1.2	BASELINE DATA: BABY'S CHARACETERISTICS		X	X
TABLE 2.1	PRIMARY ANALAYSIS OF PRIMARY OUTCOMES: NEC, SEPSIS AND DEATH		X	X
TABLE 3.1	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: COMPOSITE RESULTS		X	X
TABLE 3.2	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: MICROBIOLOGY		X	X
TABLE 3.3	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: MICROBIALS		X	X
TABLE 3.4	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: COLONISATION OF STOOLS		X	X
TABLE 3.5	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: TIME TO FIRST FULL FEED		X	X
TABLE 3.6	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: OTHER DIAGNOSES		X	X
TABLE 3.7	PRIMARY ANALYSIS OF SECONDARY OUTCOMES: HOSPITAL STAY		X	X
TABLE 4.1	ADHERENCE TO PROTOCOL		X	
TABLE 4.2	ADHERENCE TO TRIAL INTERVENTION		X	
TABLE 4.3	ADHERENCE TO TRIAL INTERVENTION BY GESTATIONAL AGE		X	
FIGURE 2	ADHERENCE TO TRIAL INTERVENTION BU GESTATIONAL AGE		X	
TABLE 4.3	HARMS		X	
TABLE 4.4	INVESTIGATOR UNBLINDING		X	
TABLE 5.1	SUBGROUP ANALYSIS		X	
FIGURE 3	FOREST PLOT OF SUBGROUP ANALYSIS		X	
TABLE 5.2	BABY'S OUTCOME		X	
TABLE 6	OTHER OUTCOME DATA COLLECTED		X	
TABLE 7	NECOROTISING ENTEROCOLITIS		X	
TABLE 8	POST NATAL AGE AT RANDOMISATION		X	
TABLE 9	COLONISATION WITH *B. BREVE* BBG AT 2 WEEKS POST NATAL AGE VS 36 WEEKS POST MENSTRUAL AGE		All	
TABLE 10	SIGNIFICANCE OF DETERMINANTS OF SUCCESSFUL COLONISATION WITH *B. BREVE* BBG	*B. breve* BBG treatment arm only		

PiPS: Trial of probiotic administered early to prevent infection and necrotising enterocolitis (ISRCTN Number: 05511098; Eudract Number: 2006-003445-17)

FIGURE 1: PARTICIPANT FLOW



[a] Includes XX who withdrew consent
[b] Includes XX who withdrew consent

TABLE 1.1: BASELINE DATA: MOTHER'S CHARACTERISTICS (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)
F1:C6	Ethnic Group.			
	White	n(%)	XX (XX.X)	XX (XX.X)
	Indian	n(%)	XX (XX.X)	XX (XX.X)
	Pakistani	n(%)	XX (XX.X)	XX (XX.X)
	Bangladeshi	n(%)	XX (XX.X)	XX (XX.X)
	Black African	n(%)	XX (XX.X)	XX (XX.X)
	Black Caribbean	n(%)	XX (XX.X)	XX (XX.X)
	Other	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:C3	Mother's Age (years).	n	XXX	XXX
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
F1:C7	Antenatal Steroid Use.			
	Yes, <24 hours before birth.	n(%)	XX (XX.X)	XX (XX.X)
	Yes, ≥24 hours before birth.	n(%)	XX (XX.X)	XX (XX.X)
	None	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:C8	Membrane Rupture > 24 hours before birth.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:C9	Chorioamnionitis in 24 hours before birth.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:C10	Antibiotics in 24 hours before birth.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX

TABLE 1.2: BASELINE DATA: BABY'S CHARACTERISTICS (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)
	Enrolling Centre			
	Centre 1	n(%)	XX (XX.X)	XX (XX.X)
	Centre 2	n(%)	XX (XX.X)	XX (XX.X)
	Centre 3	n(%)	XX (XX.X)	XX (XX.X)
	Centre 4	n(%)	XX (XX.X)	XX (XX.X)
	Centre 5	n(%)	XX (XX.X)	XX (XX.X)
	Centre 6	n(%)	XX (XX.X)	XX (XX.X)
	Centre 7	n(%)	XX (XX.X)	XX (XX.X)
	Centre 9	n(%)	XX (XX.X)	XX (XX.X)
	Centre 10	n(%)	XX (XX.X)	XX (XX.X)
	Centre 11	n(%)	XX (XX.X)	XX (XX.X)
	Centre 12	n(%)	XX (XX.X)	XX (XX.X)
	Centre 13	n(%)	XX (XX.X)	XX (XX.X)
	Centre 19	n(%)	XX (XX.X)	XX (XX.X)
	Centre 21	n(%)	XX (XX.X)	XX (XX.X)
	Centre 22	n(%)	XX (XX.X)	XX (XX.X)
	Centre 23	n(%)	XX (XX.X)	XX (XX.X)
	Centre 24	n(%)	XX (XX.X)	XX (XX.X)
	Centre 25	n(%)	XX (XX.X)	XX (XX.X)
	Centre 26	n(%)	XX (XX.X)	XX (XX.X)
	Centre 39	n(%)	XX (XX.X)	XX (XX.X)
	Centre 40	n(%)	XX (XX.X)	XX (XX.X)
	Centre 41	n(%)	XX (XX.X)	XX (XX.X)
	Centre 42	n(%)	XX (XX.X)	XX (XX.X)
	Centre 43	n(%)	XX (XX.X)	XX (XX.X)
RN data	Age at Randomisation (hours)	Median	XX.X	XX.X
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	<24 hours	n(%)	XX (XX.X)	XX (XX.X)
	24 to <48 hours	n(%)	XX (XX.X)	XX (XX.X)
	>48 hours	n(%)	XX (XX.X)	XX (XX.X)
F1:A1, A2	Gestational Age (weeks) at birth	Median	XX.X	XX.X
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	23 to <24 weeks	n(%)	XX (XX.X)	XX (XX.X)
	24 to <25 weeks	n(%)	XX (XX.X)	XX (XX.X)
	25 to <26 weeks	n(%)	XX (XX.X)	XX (XX.X)
	26 to <28 weeks	n(%)	XX (XX.X)	XX (XX.X)
	28 to <30 weeks	n(%)	XX (XX.X)	XX (XX.X)
	≥30 weeks	n(%)	XX (XX.X)	XX (XX.X)
F1:A3	Sex			
	Male	n(%)	XX (XX.X)	XX (XX.X)
	Female	n(%)	XX (XX.X)	XX (XX.X)
	Indeterminate	n(%)	XX (XX.X)	XX (XX.X)

Table 1.2: Continued

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)
F1:A4	Singleton	n(%)	XX (XX.X)	XX (XX.X)
	Multiple	n(%)	XX (XX.X)	XX (XX.X)
F1:D8	Babies born.			
	1	n(%)	XX (XX.X)	XX (XX.X)
	2	n(%)	XX (XX.X)	XX (XX.X)
	3	n(%)	XX (XX.X)	XX (XX.X)
	≥ 4	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:C1	Born in enrolling hospital.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:D4	Mode of delivery.			
	Vaginal birth	n(%)	XX (XX.X)	XX (XX.X)
	Caesarean before labour onset	n(%)	XX (XX.X)	XX (XX.X)
	Caesarean after labour onset	n(%)	XX (XX.X)	XX (XX.X)
	Other	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
	Forceps or ventouse used.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:D6	Main cause of preterm birth.			
	Pre labour rupture of membranes	n(%)	XX (XX.X)	XX (XX.X)
	Preterm labour	n(%)	XX (XX.X)	XX (XX.X)
	APH	n(%)	XX (XX.X)	XX (XX.X)
	PIH	n(%)	XX (XX.X)	XX (XX.X)
	Other maternal illness	n(%)	XX (XX.X)	XX (XX.X)
	Poor fetal growth (mother well)	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:D7	Birth weight. (g)	n	XXX	XXX
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Birth weight z-score.	n	XXX	XXX
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
F1:D9	Heart rate >100bpm 5 minutes after birth.			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX

Table 1.2: Continued

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)
F1:D11	Apgar score 5 minutes after birth.			
	1-3	n(%)	XX (XX.X)	XX (XX.X)
	4-6	n(%)	XX (XX.X)	XX (XX.X)
	7-10	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F1:D12	Baby's worst base excess in 1 hour after birth.			
		n	XXX	XXX
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
F1:D10	Temperature on admission to neonatal unit.			
		n	XXX	XXX
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	CRIB II[a]	n	XXX	XXX
		Mean(StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
F3:B6	Any Congenital Malformations			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
	If Yes:			
	type 1	n(%)	XX (XX.X)	XX (XX.X)
	type 2	n(%)	XX (XX.X)	XX (XX.X)
	type 3	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F2	Post natal age at first feed (days)[b]	n	XXX	XXX
		Mean(StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
		Median	XX.X	XX.X
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
		Missing	XX	XX
F2	Type of milk received (0 to 14 days)[b]			
	Maternal breast milk	n(%)	XX (XX.X)	XX (XX.X)
	Donor breast milk	n(%)	XX (XX.X)	XX (XX.X)
	Formula	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
	Maternal breast milk only (0 to 14 days)[b]			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX

[a] Parry et al. Lancet 2003; 361: 1789-91
[b] These data were collected post randomisation.

TABLE 2.1: PRIMARY ANALYSIS OF PRIMARY OUTCOMES: NEC, SEPSIS AND DEATH (INTENTION TO TREAT)

	B. breve BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio (95% CI)
Primary Analysis[a]			
Sepsis[b]	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
NEC[c]	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Death	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] Adjusted for centre, sex, gestational age at birth, randomisation < 24 hours of age. Clusters for correlations between multiple births are accounted for.
[b] Sepsis is defined as blood stream infection with non-skin commensals after 72 hours post natal age and < 46 weeks post menstrual age.
[c] Necrotising Enterocolitis (Bell stage II or higher)

TABLE 3.1: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: COMPOSITE RESULTS (INTENTION TO TREAT)

	B. breve BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio (99% CI)
Sepsis[a], NEC[b] or Death	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] Sepsis is defined as blood stream infection with non-skin commensals after 72 hours post natal age and < 46 weeks post menstrual age. Clusters for correlations between multiple births are accounted for.
[b] Necrotising Enterocolitis (Bell stage II or higher)

TABLE 3.2: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: MICROBIOLOGY (INTENTION TO TREAT)

Form: Q no		*B. breve* BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio / Mean difference (99% CI)
Micro Q3	Blood culture positive for skin commensal			
	Yes	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	No	XX (XX.X)	XX (XX.X)	
	Missing	XX	XX	
Micro Q2	Any blood culture taken			
	Yes	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	No	XX (XX.X)	XX (XX.X)	
	Missing	XX	XX	
Micro Q2	Number of blood cultures taken per baby			
	n	XXX	XXX	
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
	0	XX (XX.X)	XX (XX.X)	
	1	XX (XX.X)	XX (XX.X)	
	2	XX (XX.X)	XX (XX.X)	
	3	XX (XX.X)	XX (XX.X)	
	4	XX (XX.X)	XX (XX.X)	
	5	XX (XX.X)	XX (XX.X)	
	6	XX (XX.X)	XX (XX.X)	
	7	XX (XX.X)	XX (XX.X)	
	8	XX (XX.X)	XX (XX.X)	
	9	XX (XX.X)	XX (XX.X)	
	≥ 10	XX (XX.X)	XX (XX.X)	
	Missing	XX	XX	

Table 3.2 Continued

Form: Q no		*B. breve* BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio / Mean difference (99% CI)
Micro Q5, Q8	Antibiotic resistant blood stream infection			
	MRSA[a]	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	VRE[b]	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	ESBL[c]	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Micro Q5	Blood stream infection			
	Enterobacteriaceae	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Enterococcus	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Staphylococcus	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Fungi	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Non-skin	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Micro Q10	Isolates of organisms from normally sterile site (other than blood)			
	Site 1	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Site 2	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	…			
	Site n	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Micro Q13	*B. breve* BBG culture from normally sterile site			
	Site 1	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Site 2	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	…			
	Site n	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] methicillin-resistant *Staphylococcus aureus*
[b] vancomycin resistant enterococci
[c] extended spectrum betalactamase producing Gram negative bacteria

TABLE 3.3: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: ANTIMICROBIALS[A] (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)	Risk Ratio/ Mean difference (99% CI)
F3:B8 F2	Total days of antibiotics (post 72 hours)	n	XXX	XXX	
		Mean(StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	
F3:B8 F2	Total days of antifungals for treatment (post 72 hours)	n	XXX	XXX	
		Mean(StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	

[a] Contrary to the protocol, antimicrobials were collected and reported from birth until the earlier of hospital discharge, death or database lock.

TABLE 3.4: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: COLONISATION OF STOOLS (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio (99% CI)
2 weeks				
Culture				
B. breve BBG	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
MRSA[a]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
VRE[b]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
ESBL[c]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
PCR				
B. breve BBG	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
MRSA[a]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
VRE[b]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
ESBL[c]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
36 weeks pma				
Culture				
B. breve BBG	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
MRSA[a]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
VRE[b]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
ESBL[c]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
PCR				
B. breve BBG	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
MRSA[a]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
VRE[b]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
ESBL[c]	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] methicillin-resistant *Staphylococcus aureus*
[b] vancomycin resistant enterococci
[c] extended spectrum betalactamase producing Gram negative bacteria

TABLE 3.5: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: TIME FROM BIRTH TO FIRST FULL FEED (POST NATAL AGE), (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)	Adjusted 99% CI
F3:B3	Reached full feeds				
	Yes	n(%)	XXX (XX.X)	XXX (XX.X)	
	Censored - due to death	n(%)	XXX (XX.X)	XXX (XX.X)	
	Censored - due to discharge	n(%)	XXX (XX.X)	XXX (XX.X)	
F3:B3	Post natal age at first full feed	Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		99% CI	(XX.X to XX.X)	(XX.X to XX.X)	XX.X (XX.X to XX.X)
	Change in weight z-score (from baseline to 36 weeks pma)				
		n	XXX	XXX	
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	

TABLE 3.6: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: OTHER DIAGNOSES (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk ratio (99% CI)
F3:C2	Bronchopulmonary Dysplasia at 36 weeks				
	Yes	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	
	If Yes;				
	Moderate	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Severe	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	Missing	n	XX	XX	
F3:B4	Hydrocephalus and / or intraparenchymal cysts[a]				
	Yes	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	
F3:B7	Any Retinopathy of Prematurity (ROP) Examination				
	Yes	n(%)	XX (XX.X)	XX (XX.X)	
	No	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	
	If yes, ROP present				
	Yes	n(%)	XX (XX.X)	XX (XX.X)	
	No	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	
	If yes, worst Stage of ROP				
	1	n(%)	XX (XX.X)	XX (XX.X)	
	2	n(%)	XX (XX.X)	XX (XX.X)	
	3	n(%)	XX (XX.X)	XX (XX.X)	
	4	n(%)	XX (XX.X)	XX (XX.X)	
	5	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	
F3:B7	Treated for ROP				
	Yes	n(%)	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)	
	Missing	n	XX	XX	

[a] Intraparenchymal cyst defined as any cyst within the parenchyma and includes both porencephaly and cystic periventricular leucomalacia.

TABLE 3.7: PRIMARY ANALYSIS OF SECONDARY OUTCOMES: HOSPITAL STAY (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)	*Mean Difference (99% CI)*
dates	Total hospital stay	n	XXX	XXX	
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	
F3:B10	Intensive care	n	XXX	XXX	
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	
F3:B10	High-dependency care	n	XXX	XXX	
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	
	Special care	n	XXX	XXX	
		Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)	XX.X (XX.X to XX.X)
		Median	XX.X	XX.X	
		(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)	
		(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)	
		Missing	XX	XX	

XX babies were still in hospital at the time of analysis.

TABLE 4.1: ADHERENCE TO PROTOCOL (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
Randomisation > 48 hours post-natal age	n(%)	XX (XX.X)	XX (XX.X)
Gestational age < 23^{+0} weeks	n(%)	XX (XX.X)	XX (XX.X)
Gestational age ≥ 30^{+6} weeks	n(%)	XX (XX.X)	XX (XX.X)

TABLE 4.2: ADHERENCE TO TRIAL INTERVENTION (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
First dose given			
Yes	n(%)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX
Post menstrual age at first dose (weeks)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Post menstrual age at last dose (weeks)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Total Duration (days)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Total Duration of interruption(s) (days)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Percent of recommended doses taken [a]	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Permanent early discontinuation	n(%)	XX (XX.X)	XX (XX.X)
Reason for permanent early discontinuation			
Parental request	n(%)	XX (XX.X)	XX (XX.X)
Clinician recommendation	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX

[a] randomisation to 36 weeks post menstrual age. Proportions will be > 100% if the more doses than recommended were taken.

TABLE 4.3 ADHERENCE TO TRIAL INTERVENTION BY GESTATIONAL AGE AT BIRTH (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
Percent of recommended doses taken [a]			
23 weeks gestational age	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
24 weeks gestational age	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
25 weeks gestational age	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
26 - 27 weeks gestational age	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
28 - 30 weeks gestational age	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX

[a] randomisation to 36 weeks post menstrual age. Proportions will be > 100% if the more doses than recommended were taken.

FIGURE 2 ADHERENCE TO TRIAL INTERVENTION BY GESTATIONAL AGE (INTENTION TO TREAT)

This figure will present a plot of error bars for adherence as calculated according to section 7.3, by gestational age group, by intervention arm.

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TABLE 4.3 HARMS (INTENTION TO TREAT)

Event		*B. breve* BBG (n=XXX)	Placebo (n=XXX)
XXXXXXXXXXXXXXXXXXXXXXXXXX	n(%)	XX (XX.X)	XX (XX.X)
XXXXXXXXXXXXXXXXXXXXXXXXXX	n(%)	XX (XX.X)	XX (XX.X)
XXXXXXXXXXXXXXXXXXXXXXXXXX	n(%)	XX (XX.X)	XX (XX.X)

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TABLE 4.4: INVESTIGATOR UNBLINDING (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
Investigator unblinded	n(%)	XX (XX.X)	XX (XX.X)

TABLE 5.1: SUBGROUP ANALYSIS (INTENTION TO TREAT)

	B. breve BBG (n=XXX)	Placebo (n=XXX)	Adjusted Risk Ratio (95% CI)
Sepsis			
By Gestational Age, p=0.XXXX			
23 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
24 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
25 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
26-27 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
28-30 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Sex, p=0.XXXX			
Male	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Female	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Randomisation time, p=0.XXXX			
< 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
≥ 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By *B. breve* BBG Colonisation, p=0.XXXX			
Yes	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
No	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
NEC			
By Gestational Age[b], p=0.XXXX			
23 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
24 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
25 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
26-27 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
28-30 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Sex, p=0.XXXX			
Male	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Female	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Randomisation time, p=0.XXXX			
< 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
≥ 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By *B. breve* BBG Colonisation, p=0.XXXX			
Yes	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
No	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] Adjusted for sex, gestational age at birth, randomisation < 24 hours of age. Clusters for correlations between multiple births are accounted for.
[b] Effect of increasing gestational age by 1 week is XX.X (XX.X to XX.X).

Table 5.1 Continued

	***B. breve* BBG (n=XXX)**	**Placebo (n=XXX)**	**Adjusted Risk Ratio (95% CI)**
Death			
By Gestational Age, p=0.XXXX			
23 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
24 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
25 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
26-27 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
28-30 weeks	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Sex, p=0.XXXX			
Male	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
Female	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By Randomisation time, p=0.XXXX			
< 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
≥ 24 hours	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
By *B. breve* BBG Colonisation, p=0.XXXX			
Yes	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)
No	XX (XX.X)	XX (XX.X)	XX.X (XX.X to XX.X)

[a] Adjusted for sex, gestational age at birth, randomisation < 24 hours of age. Clusters for correlations between multiple births are accounted for.

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FIGURE 3: FOREST PLOT OF SUBGROUP ANALYSIS (INTENTION TO TREAT)

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TABLE 5.2: BABY'S OUTCOME (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
In hospital	n(%)	XX (XX.X)	XX (XX.X)
Discharged Home	n(%)	XX (XX.X)	XX (XX.X)
Death	n(%)	XX (XX.X)	XX (XX.X)
Respiratory failure	n(%)	XX (XX.X)	XX (XX.X)
Congenital malformation	n(%)	XX (XX.X)	XX (XX.X)
Brain injury	n(%)	XX (XX.X)	XX (XX.X)
Infection	n(%)	XX (XX.X)	XX (XX.X)
NEC	n(%)	XX (XX.X)	XX (XX.X)
Other gut pathology	n(%)	XX (XX.X)	XX (XX.X)
Other	n(%)	XX (XX.X)	XX (XX.X)

TABLE 6: OTHER OUTCOME DATA COLLECTED (INTENTION TO TREAT)

Form: Q no			*B. breve* BBG (n=XXX)	Placebo (n=XXX)
F3:B4	Cerebral Ultrasound Scan performed			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F3:B4	Cerebral Ultrasound Results			
	No abnormal results	n(%)	XX (XX.X)	XX (XX.X)
	IVH	n(%)	XX (XX.X)	XX (XX.X)
	HPI	n(%)	XX (XX.X)	XX (XX.X)
	Hydrocephalus	n(%)	XX (XX.X)	XX (XX.X)
	Porencephalic cyst	n(%)	XX (XX.X)	XX (XX.X)
	Periventricular leucomalacia	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F3:B5	Treatment for patent ductus			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F3:B5	If Yes;			
	Indometacin or ibuprofen	n(%)	XX (XX.X)	XX (XX.X)
	Surgical Ligation	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
	Any positive blood culture			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX
F3:C2	Post natal corticosteroids			
	Yes	n(%)	XX (XX.X)	XX (XX.X)
	No	n(%)	XX (XX.X)	XX (XX.X)
	Missing	n	XX	XX

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TABLE 7: NECOROTISING ENTEROCOLITIS (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
Number of episodes of NEC stage ≥ II			
None	n(%)	XX (XX.X)	XX (XX.X)
1	n(%)	XX (XX.X)	XX (XX.X)
2	n(%)	XX (XX.X)	XX (XX.X)
3	n(%)	XX (XX.X)	XX (XX.X)
4 or more	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX
Worse stage NEC			
Stage I	n(%)	XX (XX.X)	XX (XX.X)
Stage II A or B	n(%)	XX (XX.X)	XX (XX.X)
Stage III A	n(%)	XX (XX.X)	XX (XX.X)
Stage III B	n(%)	XX (XX.X)	XX (XX.X)
Post menstrual age at first NEC	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Surgery for any NEC			
No	n(%)	XX (XX.X)	XX (XX.X)
Peritoneal drainage alone	n(%)	XX (XX.X)	XX (XX.X)
Laparotomy, no enterostomy	n(%)	XX (XX.X)	XX (XX.X)
Laparotomy, with enterostomy	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX
Death due to any NEC			
Yes	n(%)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX
Spontaneous intestinal perforation			
Yes	n(%)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)
Missing	n	XX	XX

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TABLE 8: POST NATAL AGE (INTENTION TO TREAT)

		B. breve BBG (n=XXX)	Placebo (n=XXX)
Post natal age at randomisation (days)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX
Post natal age at first dose (days)	n	XXX	XXX
	Mean (StdDev)	XX.X (XX.XX)	XX.X (XX.XX)
	Median	XX.X	XX.X
	(Q1 to Q3)	(XX.X to XX.X)	(XX.X to XX.X)
	(Min to Max)	(XX.X to XX.X)	(XX.X to XX.X)
	Missing	XX	XX

TABLE 9: COLONISATION WITH *B. BREVE* BBG AT 2 WEEKS POST NATAL AGE VS 36 WEEKS POST MENSTRUAL AGE (INTENTION TO TREAT)

		Colonised with *B. breve* BBG at 2 weeks post natal age		
		Yes	No	Total
***B. breve* BBG**				
Colonised with *B. breve* BBG 36 weeks post menstrual age				
Yes	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Total	n	XX	XX	XX
Placebo				
Colonised with *B. breve* BBG 36 weeks post menstrual age				
Yes	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Total	n	XX	XX	XX
Total				
Colonised with *B. breve* BBG 36 weeks post menstrual age				
Yes	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
No	n(%)	XX (XX.X)	XX (XX.X)	XX (XX.X)
Total	n	XX	XX	XX

TABLE 10: SIGNIFICANCE OF DETERMINANTS OF SUCCESSFUL COLONISATION WITH *B. BREVE* BBG (INTENTION TO TREAT)

	Single Factor Models	Model 2	Model 3	…	Model N
Parameter 1	XX.X, 0.XXXX		XX.X, 0.XXXX		XX.X, 0.XXXX
Parameter 2	XX.X, 0.XXXX	XX.X, 0.XXXX	XX.X, 0.XXXX		XX.X, 0.XXXX
Parameter 3	XX.X, 0.XXXX				
Parameter 4	XX.X, 0.XXXX	XX.X, 0.XXXX	XX.X, 0.XXXX		XX.X, 0.XXXX
Parameter 5	XX.X, 0.XXXX				
Parameter 6	XX.X, 0.XXXX				XX.X, 0.XXXX

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