Standard Operating Procedure: Use of Data Analysis Macro after SeptiFast PCR	
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Author: …….	Approved by: …….
Signature:	Signature:
Date:	Date:

Introduction and Purpose

This SOP details the procedures specified by Roche Diagnostics for the analysis of the data automatically stored following the Septi*Fast* PCR. This involves a process of manual editing and analysis of the PCR data using Light Cycler 2.0 software (version 4.1) and export to the SeptiFast Identification Software (SIS). This is the final step in identifying the bacterial and fungal DNA of the organisms present in patient blood samples from a list specified in the Septi*Fast* Test Master List (SML). When this is complete a report is generated showing the result and whether it is valid or not based on internal QA processes. The user instructions contained in this SOP were as supplied by the manufacturer following formal training by Roche Diagnostics of the laboratory scientists undertaking the SeptiFast assay.

When:

This SOP is used whenever the SeptiFast assay is being performed. It is taken directly from the user instructions of the CE-marked Septi*Fast* kit and must be followed **exactly** to ensure that the assay is performed according to the regulatory standard. **IMPORTANT – Before starting consult the Adverse Incidents Log located next to the LightCycler 2.0 to check for any recently identified incidents with the SeptiFast system and guidance on what to do in the event of an incident**

Who:

To be used by all users of the Septi*Fast* kit. All users must have undergone verified training by Roche Diagnostics in the use of the Septi*Fast* system. Training logs can be viewed in the Trial Master File located in the trial office A306.

Where:

This procedure will be operated within the Biomedical Facility, Clinical Sciences, Salford Royal NHS Trust.

Risk assessment
Infection
Although there is no risk of direct infection, all laboratory safety rules, safe handling of materials rules will apply.

Data Analysis

N.B. These instructions should be followed in conjunction with the appropriate diagram(s) in the Roche SeptiFast kit instruction booklet.

After the run is completed the user is prompted to edit the manual Tm values before the report can be displayed.

Pull the window down to the bottom of the screen to edit manual Tm values as described below.

The LightCycler2.0 Septi*Fast* Kit Macro contains 12 Tm calling modules (3 assays, 4 channels) as well as two Absolute Quantification modules.

The user only has to manually reprocess/edit the Tm Calling module analysis.
All Tm Calling analyses are listed in the module bar. Each analysis is named with the channel and the respective assay (G+; G- or F).

Activation of an analysis module shows all samples included in the respective analysis. Samples included are marked in the sample list. Tm values are default values. The channel and assay specific baseline value are predefined *(see baseline table in SeptiFast instruction booklet)*. A predetermined data set (multicolour compensation data), as defined by the Roche Macro, will be displayed.

General Rules for manual Tm Calling
Only Peak maxima above the following baseline values should be marked with a Tm bar - RC G- channel 610 and 670 and RC F channel 705 are exceptions *(refer to example graphs in SeptiFast instruction booklet).*

Default Tm bars have to be adjusted to the real peak maxima.

Peak maxima must be selected between channel specific valid Tm ranges *(see table in SeptiFast instruction booklet).*

Default Tm bars are deactivated if no peak is detected or if a peak is below the baseline.

How to perform manual Tm analysis.
Start with the first analysis in the module bar by clicking on the analysis icon.

Melting Peaks are shown in the image on the lower right side

Open the view for the melting peak window and the table containing Tm values by pulling the flexible screen positioner.

Mark all sample positions that correspond to the chosen analysis from the module bar. (e.g. All G(+) samples if performing an analysis for gram positive results.

Mark only the respective RC [e.g. G(+) #RC#] and adjust the default peak value by pulling the bar to the peak

Delete the Tm bars that are not used

Mark the RC and the NC together to evaluate whether the NC is below the baseline

Mark only the NC and deactivate all bars if there is no peak as expected

If a peak above the baseline is observed, adjust the bar as described for the RC.

Proceed with each sample as described for the NC.

After the first analysis module is complete, proceed with the remaining Tm analysis modules in the same way.

At the end of the Tm Calling (for a specific capillary), there must be only peak maxima with Tm bar.

Low concentrations of *Enterococcus faecium* (G+, CH 705, Tm 51.8 – 55.9) and *Candida albicans* (F, CH 640, Tm 53.4 – 57.5) may appear as shoulders of the respective IC peaks (G+, CH 705, Tm 45.5 – 49.6; F, CH 640,Tm 44.8 – 48.0). Mark these shoulders with Tm bars at Tm 53.9 (G+, CH 705) and Tm 55.5 (F, CH 640).

After the manual Tm analyses are finished, press <Finish> in the wizards' message window. The file is saved automatically. The Report can be viewed on the Front Screen. Print out two copies of the report and close it afterwards.

Septi*Fast* Identification Software (SIS)

For final automated analysis with the Septi*Fast* Identification Software proceed as described in the following.

Export the *.ixo file from the LightCycler SW 4.1 using the <File>,<Export>,<.ixo File> function from the menu bar. Select the target folder <C:\Export to SIS> to store the .ixo file.

Do not change the name of your experiment.

Activate the SIS by double clicking on the desktop icon.

The <Start> button opens the .ixo file saved last.

Select "Yes" or "No" for the decision whether the LightCycler® Report contains the flag <User Developed or Modified Test Method>

The result sheet for the experiment contains the following information:

(refer to figures in SeptiFast instruction booklet)

1. Information about invalid samples/assays or runs [marked with a red cross]

2. Information about valid samples/assays or runs containing the data about the detected peak and the corresponding analyte from the Septi*Fast* Master List (SML).

3. Information about valid samples containing no analyte [marked with a θ].

Print three copies of the SIS Report – First copy to be appended to the appropriate LCRF.

Second copy to be kept in a SeptiFast data master file along with the report printed above in the trial office.

Third copy to be kept along with the report printed above in the 'SeptiFast Study' file near the Light Cycler 2.0.

Cross verification of the software analysis

The software analysis performed as above should be verified by a second person. However raw data is not available for a second analysis from the same test run because the SeptiFast test is processed via a macro. The following method is to be used for analysis verification.

The person responsible for the analysis will unmark all the Tm calling bars and invite the second person to do an independent analysis. This second analysis is saved with the second operator's name added to the initial file name. All the *.ixo* files should be stored in the C:\ drive with in the folder name 'Export to SIS'.

Once the second analysis is complete, the person responsible signs the laboratory CRF and attaches a copy of the analysis to the laboratory CRF.