Standard Operating Procedure: Setting up PCR for SeptiFast run.	
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Author: ………… Approved by: …………

Signature: Signature:

Date: Date:

Purpose
This SOP details the procedures specified by Roche Diagnostics for the setting up of the Septi*Fast* PCR run using the DNA extract prepared from blood (see S/Fast #11). The PCR will amplify, detect and identify bacterial and fungal DNA of the microorganisms list specified in the Septi*Fast* Test Master List (SML) present in the extract using the LightCycler® 2.0 Instrument. 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
All blood samples sent to the laboratory should be considered potentially infectious. All laboratory safety rules apply and laboratory coats/gowns and gloves must be worn, as appropriate.

Chemical
Chemicals harmful by inhalation, ingestion and skin; also by direct contact with skin and eyes. If splash occurs to skin wash with water, and if to eyes use Eye Wash Station fluid.

Equipment/materials required
SeptiFast PCR preparation cooling block
Disposable gloves

DNA free tips M^{GRADE}
• 20µL Filter Tips M^{GRADE} • 100µL Filter Tips M^{GRADE}
• 1 mL Filter Tips M^{GRADE}

Pipets M^{GRADE}
• 1 mL Serum Pipette • Pipette 100-1000L • 2x Reference 10-100µL

Working cabinets
PCR-workstation (Labcare)

DNA decontamination reagent – e.g. LTK-008™ solution; Biodelta GmbH

Before you start

- Clean and decontaminate the laminar flow box and the PCR workstation thoroughly using e.g. LTK-008™ solution; Biodelta GmbH.
- Turn on laminar flow box and air stream for at least 30 min.
- Turn on PCR workstation (UV-light) for at least 30 min
- Turn off UV-light while working.
- Thaw the LightCycler2.0 Septi*Fast* Kit reagents at 2-8°C in the Septi*Fast* Cooling Block. (this should be done during DNA extraction)
- Mix gently and centrifuge briefly.
- Prepare all consumables, tools (pipettes) and reagents needed and a waste container under the PCR workstation and arrange them in a suitable way.

DNA from the target organisms of the LightCycler® Septi*Fast* Test is present in the environment. In order to avoid false positive results by detection of such as environmental DNA (DNA contamination) it is necessary to establish a workflow free of contaminating DNA. In particular the operator may be a source of DNA contamination, since the human skin and upper respiratory tract are populated with different microorganisms which are also target organisms of the Septi*Fast* Test [e.g. *Streptococci* and Coagulase-Negative *Staphylococci* (CoNS)].

Always use M^{GRADE} consumables such as filter tips, tubes.

Clothes and Gloves
Wear powder-free gloves and a lab coat during the entire workflow.
Avoid exposure of skin, wear the gloves over the sleeves of the lab coat.
Change gloves immediately if contaminated or treat with DNA decontaminating reagent (e.g. LTK-008™ solution; Biodelta GmbH), gloves must be resistant to the reagent.
Do not touch the palm and fingers of gloves when putting them on.

Care of PCR workstation
Clean surfaces of workspace in the PCR workstation with DNA decontamination reagent after each PCR setup.
Clean all surfaces inside the PCR workstation in regular time intervals.
Turn on UV-light for at least 30 minutes before working.

Make sure UV-light is turned off while working.

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Treatment of Devices and Consumables

Leave specific consumables and devices used for the Septi*Fast* Test in the laminar flow, if possible.
Irradiate all items for at least 30 minutes with UV light before starting the procedure.
Use DNA free disposables only.
Use them once only.
Use Capping Tool to close capillaries (for handling instructions please refer to the LightCycler® 2.0 Instrument Operator's Manual).
In case of capillary breakage please refer to the Operator's Manual of the LightCycler® 2.0 Instrument (Chapter: Maintenance).
Briefly vortex thawed reagents (except RM 1a) and spin down)if precipitates have formed, warm the solution at 37°C for 5 minand mix gently until dissolved.

Precautions during Pipetting

Open the reagent vials, pipette tip boxes and capillary boxes only under the laminar flow / PCR workstation.

Do not use the same pipette tips and capillaries outside the laminar flow/PCR workstation.

Do not touch rim or threads of an open vial.

Arrange all items used during the procedures under the laminar flow/PCR workstation in a logical order (no criss-cross pipetting)

In case of spillage, finish the current working step and treat the working space immediately afterwards with DNA decontamination reagent (e.g. LTK-008™ solution ; Biodelta GmbH).

Setting up the PCR

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

Use the Septi*Fast* cooling block which has been pre-cooled.

Verify that the vials RM
1a and 1b are in the respective position

Verify that the G+/G-/F Detection Mixes (DM) are in the respective position

NOTE:
Depending on the volume of leftover Master Mix (MM) from a previous run as well as the number of samples to be analyzed in the current run, one or two vials of 1a, 1b and of the DMs are needed. MM reused as described by the left-over MM concept must be used immediately.

Place the closed RCs (G+;G- and F) in the appropriate position of the Septi*Fast* Cooling Block.

Place the NC as well as the eluates of the specimens to be analyzed in the appropriate position of the Septi*Fast* Cooling Block.

Pipetting of MM:
Example for seven samples (no left-over MM)
Pipette 2 x 600µL of vial 1b into 2 x vial 1a
Mix by pipetting up and down 3-4 times.

The resulting mix is called the Reaction Mix (RM).

Pipette 200µL of the upper RM into the first column of DM
(G+/G-/F)
Mix gently by pipetting up and down 3-4 times.
Change the tip after each pipetting step.
Pipette 200µL of the lower RM into the second column of DM
(G+/G-/F).
Mix gently by pipetting up and down 3-4 times
Change tip after each pipetting step.
This step results in two identical MM for each assay (G+; G- and F).

Combine both corresponding MM (G+ with G+; G- with G-; F with F) with a new pipette tip for each MM

Mix by pipetting up and down 3-4 times

Close the MM vials with their respective lids

Pipetting of the MM, the Samples and the Controls

The following procedures are carried out in a Class II cabinet

Place the 100µL capillaries in their respective positions in the cooling block using

tweezers.

Avoid criss-cross handling.

Transfer 50µL of the G+ MM to each capillary of the top row

Close leftover MM vial.
Change the pipette tip.
Transfer 50µL of the G- MM to each capillary of the middle row.
Close leftover MM.
Change the pipette tip.
Transfer 50µL of the F MM to each capillary of the bottom row.

Close the leftover MM vials with their respective lids and label all leftover MM vials with the number of left-over reactions and the date.

Store all three left-over MM vials at 2-8°C (Stability of leftover MM: 3d at 2-8°C).

Pipette 50µL of the specimen eluate to each capillary of the corresponding column above Start from the vial at the right side of the Septi*Fast* Cooling Block.

Change the pipette tip after each pipetting step.

Close each capillary immediately after completing the pipetting step for that capillary using the capping tool.

Close the specimen vial when the corresponding three capillaries are pipetted.

Proceed with the remaining specimen eluates as described for the first.

Pipette 50µL of the NC (blue) to each capillary of the corresponding column (second from left).

Change the pipette tip after each pipetting step.

Close each capillary immediately after completing the respective pipetting step for that capillary. Use the capping tool.

Close the NC vial when the corresponding three capillaries are pipetted.

Pipette 50µL of the respective RC (G+;G-; F) to the capillaries of the left-hand column

Change the pipette tip after each pipetting step.

Close each capillary immediately after completing the respective pipetting step for that capillary as well as each RC vial.

Transfer the capillaries to the LightCycler 2.0 Sample Carousel. Make sure not to mix up the order of the capillaries.

Start with RC G(+) at position 1 of the LightCycler 2.0 Sample Carousel. Proceed in the following order RC G(-) , RC F, NC G(+) , NC G(-) , NC F , sample1 G(+) , sample 1 G(-) etc.

Remove lab coat, change gloves and take the carousel to the Light Cycler 2.0 instrument room.

Transfer the LightCycler Sample Carousel to the LC 2.0 Carousel Centrifuge.

Centrifuge and transfer the LightCycler Sample Carousel to the LightCycler 2.0 Instrument.

Activate the LightCycler Septi*Fast* Kit Macro using the Roche Macro button on the Front Screen.

Type in the Assay Catalogue Number. Make sure you type in the right number.

Do not use the field <assay lot number>. If, you want to add the assay lot number to the run, insert information into the respective field in the sample editor (sample; <capillary view>).

The Experiment Kit Wizard starts the process. Follow the instructions of the Kit Wizard referring to the diagram shown in the SeptiFast instruction booklet

The Kit Wizard window moves to the upper left corner of the work pane.

Edit the number of samples and the sample names within the brackets.

Complete the starting process with “Start run”.

The Macro automatically starts the run.
During the Run a window indicates **“Running Experiment”.**
Do not perform any software operations while a run is proceeding.