Standard Operating Procedure: SeptiFast: Lysis of whole blood and DNA extraction.	
Version Number: 1.1	Date: 08/07/2010

Author: ……	Approved by: …….
Signature:	Signature:
Date:	Date:

Introduction and Purpose:

This SOP details the procedures specified by Roche Diagnostics for the extraction of bacterial and fungal DNA from whole blood using SeptiFast Lys Kit M^{GRADE} and SeptiFast Prep Kit M^{GRADE} kit. The extract will be subsequently used for amplifying, detecting and identifying bacterial and fungal DNA of the microorganisms specified in the Septi*Fast* Test Master List (SML) 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.

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

When:
This SOP is used whenever the SeptiFast assay is being performed within the Biomedical Facility, Clinical Sciences Building, Salford Royal NHS Trust. It is taken directly from the user instructions of the CE-marked SeptiFast 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**

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
Some chemicals may be harmful by inhalation, ingestion and skin adsorption, and also by direct contact with skin and eyes. If splashes occur to skin wash with water, and if to eyes use Eye Wash fluid located at the First Aid Station

Equipment/materials required
1.5 mL EDTA blood
Disposable gloves
Two separate lab coats
Centrifuge with swing out rotor for 15mL tubes min. 4200 g-force
• 1x Centrifuge 5810
• 2x adaptors (1pair) with inserts for 15mL tubes with conical bottoms
• 1x Swing out rotor min. 4200 x g
• MiniSpin Centrifuge Eppendorf
• 2x Thermomixer Comfort incl.
• 1x Thermo Block for 24 x 2.0mL vials and
• 1x Thermo Block for 8 x 15mL vials
• 1x Roller Mixer
• 1x Vortex mixer

DNA free tips M^{GRADE}
• 20µL Filter Tips M^{GRADE} • 100µL Filter Tips M^{GRADE}
• 1mL Filter Tips M^{GRADE} • 5mL Filter Tips M^{GRADE}

DNA free vials
• 1.5mL Tubes M^{GRADE} • 1.5mL Screw-cap Tubes

Working cabinets
Laminar Flow Box (Labcare) PCR-workstation (Labcare)

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

Before you start
- Clean and decontaminate the laminar flow box and the PCR workstation thoroughly DNA decontamination reagent e.g. LTK-008™ solution; Biodelta GmbH.
- Turn on laminar flow box UV-light and air stream for at least 30 min. Turn off UV whilst working.
- 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.
- 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.
- Turn on Ependorf Thermomixer and set to 56^{o}C for 15 min.

Proceed with the MagNA Lyser Instrument
Starting volume of whole blood = 1.5ml.
Put the blood collection tubes on a bottle roller and mix for 30min and then process immediately
Use a white pen for labelling of the Septi*Fast* Lys Kit tubes. Do not label on top.
Add 1 x 1.5 ml of whole blood per sample or Negative control (NC) into 1 Septi*Fast* Lys Kit tube.
Close Septi*Fast* Lys Kit tubes.

Transfer tubes to MagNA Lyser rotor.
Run MagNA Lyser at 7000 rpm for 70 sec.
Leave samples for approximately 10 min on the rotor stand.

Nucleic Acid Preparation
Change gloves or treat with DNA decontamination reagent e.g. LTK-008™ solution; Biodelta GmbH solution during the process as necessary.

BET1 (Blood Extraction Tube)
Pipette 150µL Proteinase K (PK) – **(*Vial 2 - Brown*)** into BET1.
Add 1mL whole blood or NC lysed with the MagNA Lyser Instrument into BET1 (use 1mL pipette).
Close BET1.
Vortex each BET immediately.
Add 10µL Internal Control (IC) directly to the whole blood or the NC (use 100 µL pipette). Pipette it on the inside wall of BET1
Add the contents of 2 vials of Lysis Buffer (LB) - **(*LB; 2 xVial 1- Orange*)**.
Close and vortex each BET immediately to ensure all contents including IC are mixed thoroughly.
Incubate for 15 min at 56°C with gentle mixing at 500 rpm in the Eppendorf Thermomixer.
Add the contents of 1 vial Binding Buffer (BB) – **(*1 x Vial 3 - Grey*)** to BET1. Close each BET immediately. Do this for all samples and then vortex BET1 tubes briefly.

BET 2
Transfer a filter column (FC) to BET2.
To avoid contamination, do not touch the lower part of the FC.
Pipette half of the specimen preparation mixture from BET 1 to the FC.
Close each BET2 immediately.
Centrifuge for 1 min at 1900 xg.
Pipette the remaining sample volume from BET1 to the FC. Use a fresh pipette tip each specimen or NC
Close each BET2 immediately. Centrifuge for 3 min at 1900 x g.

BET3
Change gloves.
Transfer the FC to BET3.
To avoid contamination, do not touch the lower part of the FC.
Add one vial Inhibition Removal Buffer (IRB) – **(V*ial 4 - Black*)** onto the FC.
Close BET3.
Centrifuge for 2 min at 4200g.
Add one vial Wash Buffer (WB) – **(*Vial 5 - Turquoise*)** onto the FC.
Close BET3 and centrifuge for 10 min at 4200 x g.
Put the Elution Buffer (EB**; *Vial 6; Colourless***) to pre-heat in the heat block at 70^0C.

BET4
Transfer the FC to BET4.
To avoid contamination, do not touch the lower part of the FC.
Close BET4.
Centrifuge for 1 min at 4200 x g.

BET5
Transfer the FC to BET5.
To avoid contamination, do not touch the lower part of the FC.

Pipette 300µL of the pre-heated Elution Buffer (EB; 70°C) directly to the centre of the FC. Close BET5.
Incubate for 5 min at room temperature.
Centrifuge for 2 min at 4200 x g.
Discard FC.
Transfer elute to 1.5mL DNA free reagent tube using a DNA free serum pipette.

Eluates may be used as templates for the LightCycler2.0 Septi*Fast* Kit immediately or stored for up to 8 days at 2-8°C or 30 days at -15 to -25°C. At 15 to 25°C elutes are stable for a maximum of 4 hours.