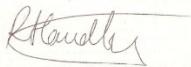


| FIEBRE Standard Operating Procedure F.15e | | | |
|---|---|--|-----------------------|
| Title | Lysis of bacterial and fungal cells for DNA | | |
| SOP Reference | Version | | Date of effect |
| F.15e | 1.1.1 | | |

SOP Development

| | Name | Title | Signature | Date |
|-----------------|---------------|---------------------------------------|--|-------------|
| Author | Becca Handley | LSHTM lab coordinator |  | 15 Nov 2019 |
| Reviewer | Ben Amos | FIEBRE microbiology consultant | | 19 Nov 2019 |
| Reviewer | James Ussher | Microbiology reference lab director | | 21 Feb 2020 |
| Approver | Heidi Hopkins | FIEBRE scientific program coordinator | [by email] | 18 Mar 2020 |

Review Tracker

| Due date for next review | Reviewer name | Signature | Date reviewed |
|--------------------------|---------------|-----------|---------------|
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Revision History

| Version No. | Effective date | Reason for change |
|-------------|----------------|-------------------|
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- 1. Title:** Lysis of bacterial and fungal cells for DNA
- 2. Purpose:** To describe the procedures for preparing bacterial microorganism DNA, from isolates that are not permitted to be shipped to the FIEBRE microbiology reference laboratory in New Zealand.
- 3. Responsible staff:** FIEBRE laboratory staff
- 4. Background & Rationale:** A blood culture will be performed for each patient enrolled in the FIEBRE study. For positive cultures, laboratory staff at the study site will identify the microorganism/s present, and will test for antimicrobial susceptibility. Staff will then ship a sample of each isolate to the reference laboratory in New Zealand, where MALDI-TOF will be used for confirmation and quality control. Some bacterial and fungal isolates are considered “unwanted organisms” in New Zealand; we are not permitted to ship these isolates to New Zealand. For these isolates, laboratory staff at study sites will extract DNA from the bacteria or fungi using a boiled lysate procedure. The DNA will be shipped to the reference laboratory for analysis with genetic methods. The list of organisms to be treated in this way will include some isolates that have only been identified to genus level and have the potential to be identified as “unwanted organisms.”
- 5. Supplies and Materials**
 - 5.1 “Unwanted” bacterial isolates – see appendix for an example list of bacteria that have been identified and are not permitted to be sent to New Zealand
 - 5.2 Non-selective media plates
 - 5.3 Sterile loops
 - 5.4 Heat block or water bath
 - 5.5 Biohazard disposal system
 - 5.6 Nitrile or latex gloves
 - 5.7 PCR grade water
 - 5.8 PCR tubes
 - 5.9 1.5 mL Eppendorf tube
 - 5.10 0.5 mL O-ringed microtubes (sterile)
 - 5.11 Centrifuge

6. Procedures:

- 6.1 Provide a complete list of the archived microorganisms to the LSHTM FIEBRE laboratory coordinator (currently Becca Handley [Rebecca.handley1@lshtm.ac.uk]) to determine which bacteria or fungi will need to be lysed for DNA. Note: the majority of bacteria isolated can be sent as cryopreserved live cultures, and only a small number will need to be lysed.
- 6.2 As the majority of the isolates that require lysis have the potential to be HG3 pathogens, all work should be carried out within the appropriate Containment Level 3 facilities.
- 6.3 Identify the isolates requiring lysis. Prepare a suitable agar plate (not blood or chocolate) and plate bacteria lightly so individual colonies can grow. Leave overnight in 37°C incubator.
- 6.4 The following day choose four representative colonies and resuspend them in 100 uL of PCR grade water in a capped PCR tube, 1.5 mL Eppendorf tube, or screw top tube. Gently resuspend; vortex if necessary to fully resuspend.
- 6.5 Heat in a heat block at 100°C for 10 minutes. If no heatblock is available use a water bath set to 100°C.
- 6.6 Transfer the entire contents of the tube to a new 1.5 mL Eppendorf tube.
- 6.7 Centrifuge for 10 minutes at >10,000g or at full speed if using a microcentrifuge. This will concentrate bacterial cells in pellets. The DNA is found in the supernatant.
- 6.8 Use a calibrated pipette to carefully remove the supernatant, making sure not to disturb the cell pellet. Dispense the supernatant into a new, sterile, 0.5 mL O-ring microtube.
- 6.9 Label this microtube clearly with the sample ID and the words “DNA from [insert microorganism name]”. For labelling, use stickers and pen that can withstand -80°C.
- 6.10 Store the microtube at -80 °C until ready to ship. Procedures for shipping are covered in SOP 15c.

Appendix:

Table of isolates that require lysis as they have the the potential to be unwanted or HG3 and cannot be identified further on-site. This is not an exhaustive list and is only based on FIEBRE data collected up to April 2020. A list of all microorganisms isolated should be checked with LSHTM FIEBRE staff before any shipping arrangements are made.

| Isolates | Reason for lysis |
|--|--|
| Identified as <i>Burkholderia pseudomallei</i> | “unwanted organism” and HG3 pathogen |
| Gram negative cocci | genus unknown, potentially “unwanted” |
| Non-lactose fermenting | “unwanted organism” and potential HG3 pathogen |
| <i>Bacillus</i> spp | “unwanted organism” and potential HG3 pathogen |
| Any unknown/un-identified | “unwanted organism” and potential HG3 pathogen |