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# **Protocol for Environmental Sampling, Processing and Culturing of Water and Air Samples for the Isolation of Slow-Growing Mycobacteria**

## **Standard operating procedure**

National Mycobacterium Reference Laboratory

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## Summary

This document outlines the work processes of environmental samples related to heater cooler units for the isolation and identification of slow growing *Mycobacteria*. The environmental samples referred to in this document are:

- water samples from heater-cooler units (used in cardiopulmonary bypass surgery)
- air samples in environments where heater cooler units are in operation (in support of cardiopulmonary bypass surgery)

This is an interim protocol generated as part of an incident response and will be finalised when more data is available.

## Safety

- mycobacterial culture must be performed by trained staff within a containment level 3 (CL3) laboratory
- laboratory procedures that give rise to aerosols must be conducted in a Class 1 biological safety cabinet (BSC)
- NaOH-NALC and 4% NaOH are irritating to skin and eyes, gloves and eye/face: protection must be worn when handling these agents
- use sealed buckets for centrifugation. After centrifugation, buckets must be opened in the BSC
- all samples and reagents must be held and transported safely in suitable racks
- all equipment must be serviced and maintained as per local protocols
- the above guidance should be supplemented with local COSHH and risk assessments

# 1. Cross-reference

This SOP should be used in conjunction with:

- local TB laboratory SOP, COSHH and risk assessments
- local procedures for calibration and maintenance of equipment

# 2. Purpose of the examination

This protocol provides guidance on how best to perform and process environmental water and air sampling, from heater-cooler units and environments where these units are in operation, in order to recover slow growing mycobacterial species.

A recent investigation from Switzerland and the Netherlands showed a possible association between *M. chimaera* (a slow growing Mycobacterium belonging to the *M. avium* complex) infections in patients who had undergone open chest surgery and contaminated heater-cooler units. Both the water in the heater-cooler machine and air samples taken while the units were in operation grew *M. chimaera*. Several possible cases of *M. chimaera* infections in cardiothoracic surgical patients have now been identified in the UK, hence the need for an environmental sampling protocol that will enable centres to provide comparable standardised results.

Water samples are usually investigated following filtration of 100ml of water and culturing the filters on selective and non-selective media for up to seven days (although samples from endoscope washer-disinfector units may be incubated for up to 28 days). This allows the recovery of fast growing mycobacterial species, non-fermenters and Gram positive organisms. However, this method is less likely to be successful in view of the slow growth of *M. chimaera*, and the likely presence of other environmental organisms in the water.

Aerosolisation is only likely to occur while heater-cooler units are in operation, so an active air sampling method which rapidly samples high volumes of air is preferred over passive methods, such as settle plates. Impaction samplers are recommended. Whichever model of sampler is used it should be calibrated to sample a certain volume of air in a given period of time and utilise standard-sized 7H11 selective agar plates. Standard sized 7H11 selective plates are currently available from three manufacturers (E&O Laboratories, Oxoid and ThermoFisher Scientific). No manufacturer currently routinely provides this media in the larger or Rodac plate formats used in some air sampling devices. If encountering difficulty in sourcing an appropriate air sampling

device, the PHE Biosafety Unit at Porton Down may be able to assist by loaning an air sampler.

## 3. Equipment and reagents

Water sampling:

- a. 2x 50ml universal containers (VWR, Cat.no. 202-0402)
- b. Centrifuge
- c. Class 1 BSC
- d. Gilson pipettes and filter pipette tips
- e. NaOH-NALC or 4% NaOH
- f. Phosphate Buffer (PB) pH 6.8
- g. Automated Liquid Culture System for Mycobacteria (eg BD MGIT 960)

Air sampling:

- a. Environmental air impaction sampling device capable of sampling a given volume of air
- b. Middlebrook 7H11 selective media plates (eg 7H11 containing polymyxin B, carbenicillin or ticarcillin, amphotericin B and trimethoprim)
- c. Incubator 35°C-37°C

## 4. Calibration and maintenance procedures

All equipment (including pipettes, centrifuges, biological safety cabinets (BSCs) etc.) must be serviced and/or calibrated on a regular basis (please refer to local protocols for appropriate calibration and maintenance of equipment).

Air samplers used should be calibrated to sample a certain quantity of air in a given period of time and utilise standard-sized 7H11 selective agar plates.

Airflow readings for Class 1 BSCs should be performed and recorded at least weekly.

## 5. Procedural steps

### 5.1 Water samples

#### 5.1.1 Sampling:

- the heater cooler machine should be connected and running for a minimum of five minutes before water sampling is performed
- ideally the water sampling should take place just prior to the machine undergoing its disinfection cycle
- sodium thiosulphate should be added to the water collection pots to neutralise hypochlorite before water sampling as per local protocol – a final concentration of at least 18mg/L is advised, but pre-dosed bottles with 20mg/L are available and acceptable
- water should be sampled from both circuits ie the 'patient' circuit and the 'cardioplegia' circuit via the tubing systems. Please ensure that sterile tubing/fittings are available for each machine being tested
- a volume of 100ml per sample is suggested
- the recommended volume of water (as per the manufacturer of the bottles) should be sampled if bottles pre-dosed with sodium thiosulphate are used
- if the water is not processed immediately, it should be stored between 2°C and 8°C for up to 24 hours

#### 5.1.2 Sample processing prior to decontamination:

1. Add 50ml of sample into each of the two sterile 50 ml universal containers
2. Centrifuge the universals at 3000xg for 30 minutes using sealed buckets within the centrifuge
3. Discard the supernatant into the liquid discard container, leaving approximately 1ml for decontamination
4. Pool the 1ml aliquots of concentrated deposit into 1x 50 ml universal for decontamination.

#### 5.1.3 Sample decontamination

Please use the method currently employed in your local laboratory. Below are two commonly used decontamination methods:

### **Decontamination using 2% sodium hydroxide (NaOH) with sodium citrate – N-acetyl-L-cysteine (NALC) decontamination method (1.5% working concentration) using 'BD BBL Mycoprep'**

NaOH: acts as a mucolytic agent and decontaminant.

NALC: acts as a mucolytic agent to allow a reduced amount of NaOH to be used.

Sodium citrate: acts to bind heavy metal ions that may be present in the specimen and which can inactivate NALC.

1. Add 4ml of NaOH-NALC to up to 2ml of sample (2:1) in a 50ml universal tube and mix by vortexing for 20 seconds, then mix gently by inverting the sealed tube several times to coat the entire inner surface of the universal tube
2. Repeat the gentle mixing only at 10 minutes during the 20 minutes decontamination time
3. After 20 minutes make each sample up to 40-45ml with sterile Phosphate Buffer (PB) pH 6.8 and mix to neutralise

### **Decontamination using 4% NaOH (modified Petroff method)**

NaOH is a commonly used decontaminant and serves as a mucolytic agent, however strict adherence to the indicated timing is required as it is still harmful to mycobacteria and over-exposure may affect culture yield.

1. Add an equal volume of 4% NaOH (0.5 N) to the sample (1:1) in a 50ml universal tube, and mix by vortexing for 20-30 seconds, then gently invert the sealed tube several times
2. Allow the NaOH to act for 15-20 minutes at room temperature (20-25°C), vortexing at regular intervals (eg every five minutes)
3. After 20 minutes make each sample up to 40-45ml with sterile Phosphate Buffer (PB) pH 6.8 and mix to neutralise

#### **5.1.4 Sample concentration:**

1. After neutralisation, centrifuge at 3000xg for 30 minutes using sealed buckets within the centrifuge
2. After centrifugation, always open centrifuge buckets in a Class 1 BSC in case of breakage
3. Discard supernatant carefully into a liquid discard container removing as much of the supernatant as possible
4. Add 1.5ml of sterile PB pH 6.8 and re-suspend deposit
5. Place samples with matched media in the universal rack then place all into the Class 1 BSC



### 5.1.5 Sample inoculation and culture:

1. Prepare and inoculate the liquid culture media bottles as per the manufacturer's instructions
2. Load the bottles onto the automated liquid culture system, and incubate for 6-8 weeks
3. Store the remaining treated deposit at -20°C in case further or repeat testing is required
4. If the samples are indicated as 'Positive' by the culture system, confirm the presence of acid fast bacilli (AFB) by performing a Ziehl-Neelsen or Auramine-phenol stain. It is recommended that a blood agar plate is set up to enable the detection of possible contamination in the culture
5. Once the presence of AFB is confirmed an aliquot of the positive culture ( $\geq 1$  ml) is to be referred to the Regional Mycobacterium Reference Laboratory as per local protocol

## 5.2 Air samples

### 5.2.1 Sampling:

1. Middlebrook 7H11 selective media plates should be inspected for evidence of contamination before use
2. Should sampling take place in theatres – even between lists – ensure testing personnel observe local dress codes
3. The sampler should be used according to the manufacturer's instructions
4. A total volume of  $1\text{m}^3$  should be sampled from each machine while it is running
5.  $0.5\text{m}^3$  of air should be sampled at a distance of approximately 0.3m from the front of the unit; a further  $0.5\text{m}^3$  should be sampled from 0.3m behind the unit
6. Additional sampling can take place at a centre's discretion but details should be recorded
7. Ensure plates are clearly labelled with the date and the area sampled

### 5.2.2 Sample culture

1. Once sampling is completed, the labelled, inoculated 7H11 plates should be incubated as soon as possible
2. Plates should be double bagged in specimen bags (to retain moisture) and incubated for up to 6 weeks at 35°C. Plates may be stored at room temperature for up to 24 hours in the event of a delay in accessing an incubator
3. The plates should be monitored weekly for growth, and any growth should undergo a Ziehl-Neelsen or Auramine-phenol stain to confirm the presence of

AFB. It is recommended that a blood agar plate is set up to enable the detection of possible contamination in the culture

4. Colony forming units should be counted where possible and reported (cfu/m<sup>3</sup>).
5. Once the presence of AFB is confirmed the colonies should be sub-cultured onto solid media and the original referred to the Regional Mycobacterium Reference Laboratory as per local protocol

## 6. Identification

Any mycobacteria identified as *M. intracellulare* by the GenoType Mycobacterium CM line probe assay (HAIN Lifescience) should be stored. In the event of cases occurring at the relevant hospital, the isolate can be retrieved and sent to the National Mycobacterium Reference Laboratory (NMRL) for further identification of *M. chimaera*.

Isolates identified by 16S rRNA gene sequencing as *M. intracellulare* or *M. chimaera* should be stored. In the event of cases occurring at the relevant hospital, the isolate can be retrieved and sent to the NMRL for further identification of *M. chimaera*.

Any isolate to be referred to the NMRL should be accompanied by the routine NMRL request form (Mycobacterium Referral-N1) which must be completed with the following details:

- referring laboratory
- sample type (Water or Air)
- referring laboratory's reference number
- initial identification result and method used (in the referring laboratory)
- 'For identification of *M. chimaera*' in the comment box

### SUMMARY OF REVISIONS

Retraining Required	No
1) Page 7 section 5.1.2: Water sample volume tested increased to 100ml	
2) Page 7 section 5.1.3: Sample decontamination section updated	
3) Page 10 section 6.0: Identification section updated to include storage of the isolates & referral of only relevant isolates.	