Public Health England

Protecting and improving the nation's health

Summary of Results
Mycobacterium spp. Scheme

External Quality Assessment for Water Microbiology

Distribution Number: **MY002**
Sample Numbers: **MY002A and MY002B**

Distribution Date:	**1 July 2019**
Results due:	**20 September 2019**
Report Date:	**17 October 2019**
Samples prepared and quality control tested by:	**Angela Appea** **Isis Asamoah** **Richard Borrill** **Margaret Njenga** **Zak Prior** **Lili Tsegaye**
Data analysed by:	**Nita Patel** **Manchari Rajkumar**
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Overview:
This unique microbiology scheme provides proficiency testing (PT) samples to laboratories that examine heater cooler unit (HCU) waters for *Mycobacterium* spp. This scheme challenges the detection and identification of this organism from this hospital water sample.

HCUs are used during open heart surgeries to warm or cool a patient as part of their care. It has recently been recognised that there is the potential for *Mycobacterium chimaera* or other species to grow in a water tank in the HCU. When the water evaporates, the mycobacteria may become dispersed into the environment as aerosols and may infect a patient during certain types of open heart surgery.

Procedure for examining samples of HCU waters for *Mycobacterium* spp. is taken from Public Health England's document 'Protocol for Environmental Sampling, Processing and Culturing of Water and Air Samples for the Isolation of Slow-Growing Mycobacteria' which can be found on this link:
Mycobacteria HCU method

Guidelines and general advice:
If you experience difficulties with any of the examinations, please refer to section 17.0 of the Scheme Guide
https://www.gov.uk/government/publications/food-and-water-proficiency-testing-schemes-scheme-guide

FEPTU Quality Control:
For homogeneity of the colony counts a minimum of 10 LENTICULE® discs, selected randomly from the batch, are examined for *Mycobacterium* spp. The FEPTU results are determined using the method in the above HTM-01-06 document.

To demonstrate homogeneity of the sample for enumeration values, a minimum of 10 LENTICULE® discs, selected randomly from a batch, are tested.

To demonstrate stability of the sample for enumeration values, a minimum of six LENTICULE discs, selected randomly from a batch, are examined throughout the distribution period.

The intended results letters provide guidance for participants regarding the assigned values.

Please contact FEPTU staff for advice and information:

Repeat samples	Carmen Gomes or Yermin Daruwalla	**Tel: +44 (0)20 8327 7119**
Data analysis	Manchari Rajkumar and Nita Patel	**Fax: +44 (0)20 8200 8264**
Microbiological advice	Zoe Prior or Nita Patel	**E-mail: foodeqa@phe.gov.uk**
General comments and complaints	Zoe Prior or Nita Patel	
Scheme Coordinator	Nita Patel	
Scheme Consultant	Nicola Elviss and Caroline Willis	

Accreditation: PHE will be applying for this scheme to be accredited with the United Kingdom Accreditation Service (UKAS) to ISO/IEC 17043:2010. However, all the quality principles in this standard have been followed to process this distribution.

A total of 26 participants were sent this distribution, of which 24 examined the samples and one did not return a result and one did not examine the samples.

Sample: MY002A

Sample type: Heater cooler waters

Request: Examine for the presence of *Mycobacterium* spp.

Contents:
Mycobacterium chimaera (47 cfu) (NCTC 13781) and *Pseudomonas fluorescens* (15 cfu) (NCTC 3756)

All levels are presented as colony forming units (cfu) per 100mL

Expected Results:

	Expected Result
Mycobacterium spp.	Detected

Number of participants reported correctly a detected result	15/24 (63%)

Sample: MY002B

Sample type: Heater cooler waters

Request: Examine for the presence of *Mycobacterium* spp.

Contents:
Mycobacterium chelonae (55 cfu) (NCTC 946) and *Burkholderia multivorans* (7 cfu) (wild strain)

All levels are presented as colony forming units (cfu) per 100mL

Expected Results:

	Expected Result
Mycobacterium spp.	Detected

Number of participants reported correctly a detected result	16/24 (67%)

Your reported result is shown in the table on page 4 of this report

Table 1: Summary of participant's results for MY002 for detection of *Mycobacterium* spp.

Lab	Results MY002A	Results MY002B
	Detected	Not detected
	Not detected	Not detected
	Detected	Detected
	Detected	Not detected
	Not detected	Detected
	Detected	Detected
	Detected	Detected
	Detected	Detected
	Detected	Not detected
	Detected	Detected
	Not detected	Detected
	Detected	Detected
	Detected	Not detected
	Not detected	Detected
	Detected	Detected
	Non-return of results	
	Detected	Not detected
	Not detected	Detected
	Not examined	
	Not detected	Detected
	Detected	Detected
	Not detected	Detected
	Not detected	Not detected
	Not detected	Detected
	Detected	Not detected
	Detected	Detected

General comments

This is the first distribution using heater cooler unit water as the sample type in the *Mycobacterium* spp. in water scheme. FEPTU will be applying for this scheme to be accredited once we have gathered more performance data.

Scheme specific comment for MY002A and MY002B

The samples in this distribution have not been scored. More data needs to be gathered before a final decision is made on how scoring will be applied for this scheme.

A breakdown of the process method used by result is shown in the table below for the 22/24 (92%) of the laboratories that provided this information.

	MY002A		MY002B	
	Membrane filtration (% correct)	BBL MGIT (% correct)	Membrane filtration (% correct)	BBL MGIT (% correct)
Detected	8/15 (53%)	5/7 (71%)	12/15 (80%)	3/7 (43%)

15/22 (70%) used the membrane filtration method to examine the samples.

The recovery of the *Mycobacterium* spp. from sample A by the participants using membrane filtration was poorer compared with the BBL MGIT system. However, for sample B recovery was better with the membrane filtration method.

A definitive conclusion on the causes of the false negative results cannot be determined due to the low number of laboratories participating in this distribution and a number of potential reasons for this. Therefore, false negative results cannot be concluded as being incorrect.

M. chelonae is fast-growing and grows well on Middlebrook agar plates (using membrane filtration). However, *M. chimera* is slower-growing and, based on the results shown here, it appears that the agar based method may not be as suitable for this, possibly due to plates drying out or becoming overgrown with other bacteria / moulds before the end of the extended incubation. The incubation temperature used for the two methods may also have an effect on the recovery of the two different organisms, since Middlebrook agar tends to be incubated at 30ºC (for Health Technical Memorandum 01-06 (HTM 01-06) but the BBL MGIT is performed at 37ºC. The number of mycobacteria in the sample may have also influenced the final results obtained by the laboratories.

Participants wishing to re-examine the samples can request a repeat sample from FEPTU.

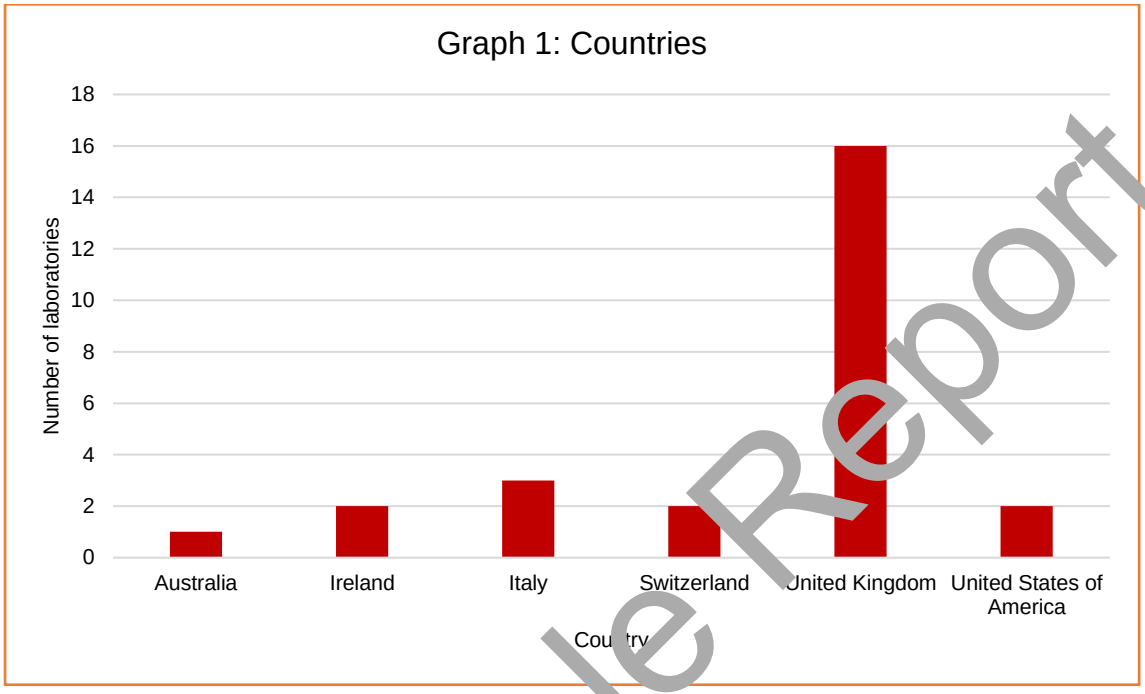
Questionnaire results:

Please note that not all participants provided the relevant information.

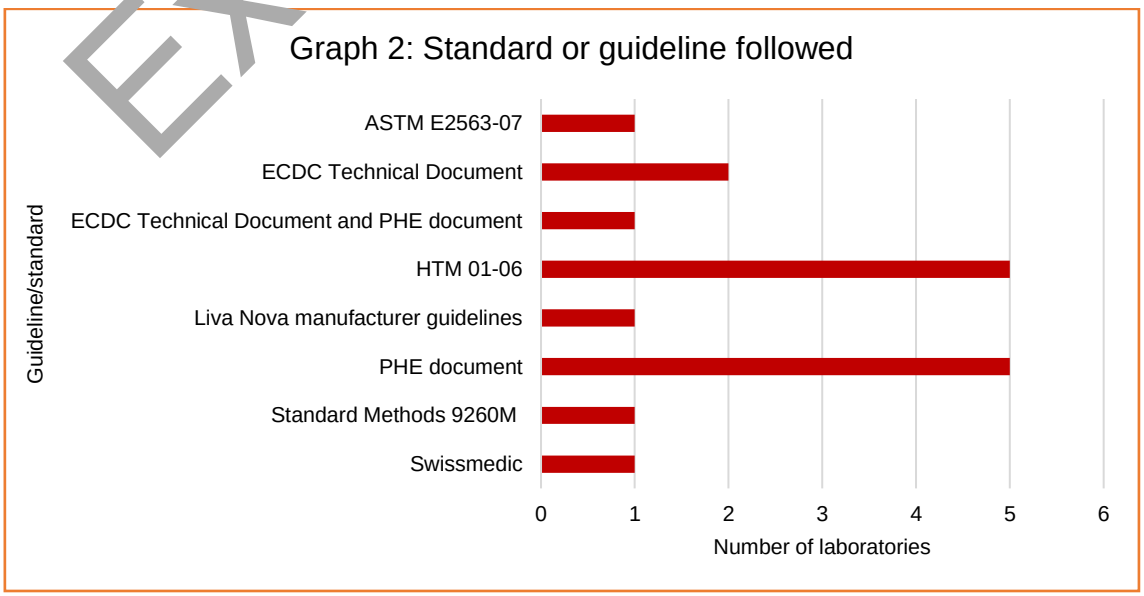
FEPTU are aware that processes are different and therefore have not attempted to categorise the information into specific groups for comparing data.

The data shown below is for information only. It does not evaluate or associate the data with a failure with PT to a method/process used nor does it attempt to compare performance of the various methods used with each other.

A total of six countries participated in this distribution (Graph 1), the majority of which were in the UK.



1. **Standard and or guideline used for the sample examination – see links to some of these documents at the end of the report**
   - Of the 24 responses received (Graph 2):
     - 5/24 (21%) used the Health Technical Memorandum 01-06 (HTM 01-06) 'Decontamination of flexible endoscopes, Part E: Testing methods
     - 6/24 (25%) used PHE's document 'Protocol for Environmental Sampling, Processing and Culturing of Water and Air Samples for the Isolation of Slow-Growing Mycobacteria'



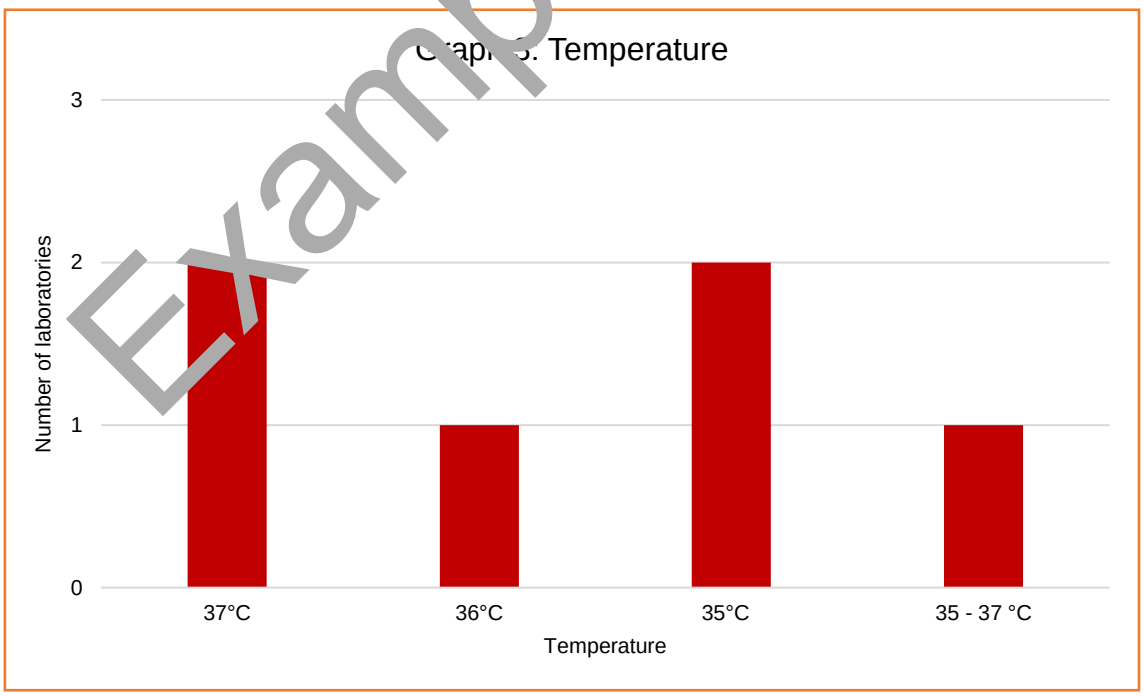
2. Examination process

- 14/20 (70%) of the laboratories examined the samples by membrane filtration followed by culture
- 6/20 (30%) of the laboratories examined the samples by BD BACTEC™ MGIT™ automated mycobacterial detection system
    - These six laboratories also centrifuged their sample prior to the decontamination step
    - Details of the chemicals, neutralisation reagents used, length of period for decontamination and volume used to inoculate the BBL MGIT tubes are shown in the table below
    - Details on the incubation temperature is shown in graph 3

Chemical used	Decontamination time (Minutes)	Neutralisation reagents used	Volume (mL)
4% Sodium hydroxide	15	Water	0.5
4% Sodium hydroxide	20	Phosphate buffer pH 6.8	0.5
4% sodium hydroxide	40	Phosphate buffered saline (PBS)	0.5
BBL Myco prep (NALC-NAOH)	20	Phosphate-buffered saline	0.5
N-acetyl-cysteine-sodium hydroxide (NALC-NaOH) contain of (4% NaOH +2.9% Tri-Na Citrate +NALC)	20	Phosphate-buffered saline	0.5
1N NaOH	30	Sterile Acidified Phosphate Buffer	2

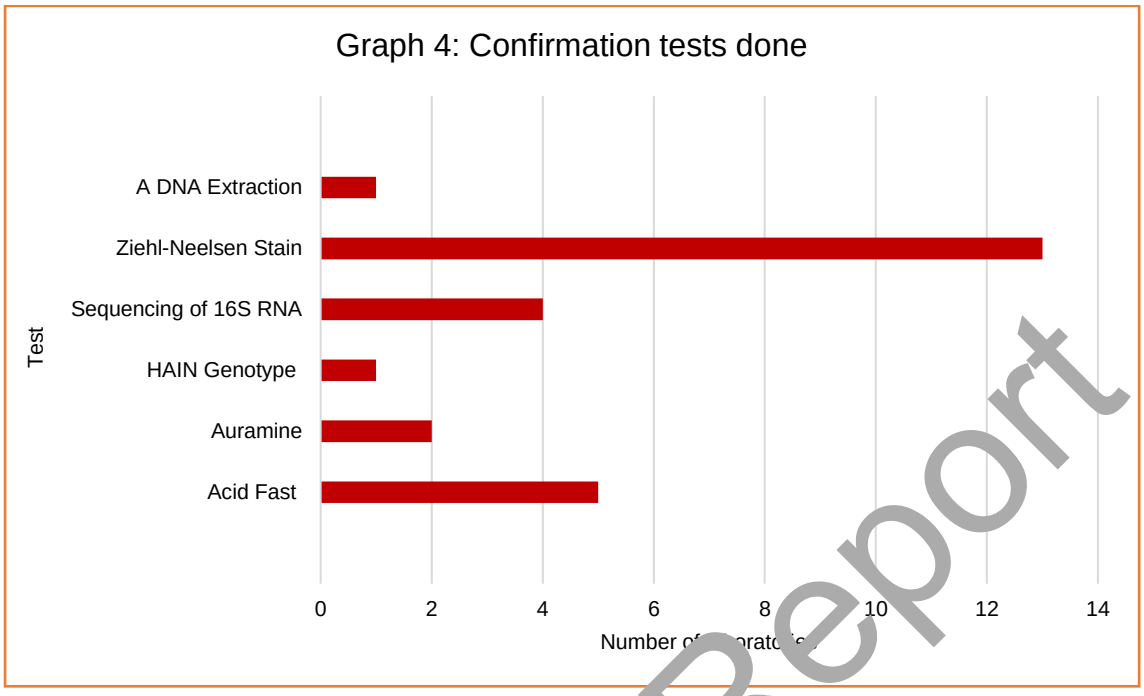
3. Temperature

Temperature used to incubate the BBL MGIT tubes is shown in graph 3. The period of incubation varied from 42 days to 56 days



4. Confirmation tests

- 22/24 (92%) of the laboratories would perform a confirmation test on presumptive *Mycobacterium* spp. isolates grown. The type of tests done are shown in graph 4
- 13/24 (54%) of the laboratories would send the isolate off to a reference laboratory



Some useful links:

ECDC Technical document:
https://www.ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/EU-protocol-for-M-chimaera.pdf

Health Technical Memorandum 01-06
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/553303/HTM01-06_PartE.pdf

Public Health England procedure for heater cooler units:
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/540325/Air_water_environmental_sampling_SOP_V2.pdf

End of report.