NHS LOTHIAN

DIRECTORATE OF LABORATORY MEDICINE

Scottish Mycobacteria Reference Laboratory

& NHS Lothian Mycobacteria Laboratory

User Manual

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Scottish Microbiology Reference Laboratories, Edinburgh:

http://www.edinburghlabmed.co.uk/Specialities/reflab/Pages/default.aspx

UKAS ISO 15189:2012 Accredited

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Scottish Mycobacteria Reference Laboratory, Scottish Reference Laboratories, Edinburgh

The Scottish Mycobacteria Reference Laboratory (SMRL) is the Scottish Reference Laboratory for Mycobacteriology, particularly for the identification, drug susceptibility testing and strain typing of *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM). The laboratory receives approximately 1900 mycobacterial cultures per year mainly from Scottish NHS laboratories. Further information regarding advice available is detailed below. SMRL is also the Scottish repository for mycobacterial cultures.

The laboratory is based within the Directorate of Laboratory Medicine, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh, EH16 4SA, UK. It is part of the Scottish Microbiology Reference Laboratories, Edinburgh and is fully integrated with the combined laboratories of NHS Lothian. SMRL is funded through an agreement between National Service Scotland and NHS Lothian. Some services have been developed which are available at a charge to the user.

This manual covers three levels of service:

1. National Reference services funded by National Services Scotland (NSS).
2. Services paid for by charging the users per test.
3. Services provided by NHS Lothian to the local and other Boards by Service Level Agreement. These Boards are currently, NHS Borders, NHS Fife, NHS Dumfries and Galloway, NHS Highland, NHS Forth Valley and NHS Tayside.

Telephone and Email Enquiries

TB Laboratory Tel: 0131 242 6022/23
TB Office Tel: 0131 242 6016
The preferred method of communication for non urgent matters is Loth.Smrl@nhs.scot
Fax machines are not considered a safe method of transmitting sensitive information; therefore, fax provision in NHS Lothian is not available.

Core Hours

The core laboratory hours are 9am to 5pm, Monday to Thursday and 9am to 4:30pm on Fridays. The laboratory is closed during weekends, Christmas and New Year holidays. At other times, Lothian laboratory users can discuss urgent AFB microscopy via the Consultant/Specialist Registrar on-call for clinical bacteriology (contact Royal Infirmary of Edinburgh switchboard Tel: 0131 536 1000). There is currently no out-of-hours AFB screening or Reference Laboratory service.

For urgent enquiries out-of-hours, the on call Consultant Microbiologist may be contacted through the RIE switchboard (Tel 0131 536 1000) in the first instance.

Contact List (Permanent Staff)

Name and Designation	Telephone Numbers and e-mail Address
For non urgent enquiries	Loth.Smrl@nhs.scot Tel 0131 242 6016
Mrs Mori Robertson, SMRL Administrator	Mori.Robertson@nhs.scot Tel: 0131 242 6016
Dr Ian Laurenson, Director	Ian.Laurenson@nhs.scot Tel: 0131 242 6079
Dr Olga Moncayo, Deputy Director	Olga.Moncayo@nhs.scot Tel: 0131 242 6070
Dr Louise Seagar, Clinical Scientist	Louise.Seagar@nhs.scot Tel: 0131 242 6009
Dr Naomi Gadsby, Consultant Clinical Scientist	Naomi.Gadsby@nhs.scot Tel: 0131 242 1260
Mr Ben Moore, Assoc. Clinical Scientist	Benjamin.Moore@nhs.scot Tel: 0131 242 6009
Mrs Pauline Claxton, BMS Team Manager	Pauline.Claxton@nhs.scot Tel: 0131 242 6009
Ms Ania Pilarska, BMS Team Manager	Anna.Pilarska@nhs.scot Tel: 0131 242 6009
Mr Daniel Garcia Puentes, BMS Team Manager	daniel.garciapuentes@nhs.scot Tel: 0131 242 6009
BMS Specialists: Ms Sandra Houston Ms Janet Maramba Mr Jack Oliver Mr Neil McMinn, MTO Mrs Rachel Rudge Mr Darren Robertson	Tel: 0131 242 6022/6023

The service is managed overall by NHS Lothian. If we are unable to deal with an issue and you wish to discuss a matter with NHS Lothian Management then contact:

Linda Mulhern
Tel: 0131 242 6017
linda.mulhern@nhs.scot
Operational Science Manager, Microbiology, NHS Lothian

Amanda Malham
Tel: 0131 242 7051
Amanda.Malham@nhs.scot
Healthcare Science Manager, Cell Sciences, NHS Lothian.

Packing and Transport of Infectious Substances

Postal Address

Scottish Mycobacteria Reference Laboratory
Scottish Microbiology Reference Laboratories, Edinburgh,
Department of Laboratory Medicine
Royal Infirmary of Edinburgh
51 Little France Crescent
Old Dalkeith Road
Edinburgh EH16 4SA

DX Address

Exchange: Edinburgh 96 EH
DX Number: DX6231201

NB Infectious substances may only be transported in packaging, which meets the U.N. Class 6.2 specifications and the 602 packing requirements. All Cultures must be sent by DX Courier or equivalent-NOT by post. They must conform to the Transport of Dangerous Goods Act. Cultures for 'routine' processing should not usually be sent in batches.

Packaging Mycobacteria Cultures or Samples:

1. Remove any water of condensation from the solid culture vial(s) - taking normal safety precautions for handling cultures.
2. Seal the cap with 'parafilm'.
3. Wrap individual vials in sufficient absorbent material to absorb all the liquid in the vial and to protect it from breakage.
4. Seal this package twice in plastic bags.

For posting by DX courier for all cultures – preferred for specimens:

1. Place the sealed specimen bag into a screw-cap container from a DX box and put with it the corresponding request forms.
2. Carefully place the destination address label provided on the green tracking label on the package, taking care not to cover any of the peel-off barcodes or tracking numbers. Also leave some space next to the lines for receiver's DX number and Exchange code. Place some clear sellotape over the label to ensure it is not easily removed or damaged by rain, again avoiding covering any peel-off labels.
3. Fill in the DX number and Exchange code of the destination.
4. Peel off **one** of the two matching tracking numbers at the bottom of the Tracked Specimen label and stick it in the provided DX logbook and fill in the receiver's details and date of dispatch.
5. At the top of the DX label, fill in the sender's name, sender's DX number, sender's exchange code and the name and contact number for the sender. This information is written on a separate card at the front of the DX logbook.
6. Place the provided blue security seal sticker over the package closure and dispatch.

For posting by First Class Post (Specimens Only):

1. Place the wrapped sample inside the inner container of the packaging and tighten the lid.
2. Place the inner container into the outer cardboard box.
3. Close the box and secure with tape.
4. Stick address label on the top of the box.
5. Stick "From" label, with senders address, on the bottom of the box.
6. Post by First Class Post or courier such as DX for samples.

Levels of Service

1. National Services Scotland funded Remit

SMRL receives cultures of presumed mycobacteria from all NHS laboratories in Scotland. These cultures are tested to determine

- Presence of mycobacteria.
- Full species identification.
- Susceptibility to standard anti-mycobacterial drugs.
- Susceptibility to second line anti-mycobacterial drugs
- Genomic comparison of *M. tuberculosis* complex strains.

2. Services available by charging users.

- Rapid liquid culture of samples from normally sterile sites (e.g. blood, CSF, bone marrow, tissue samples, aspirates and bronchoalveolar lavage samples).
- Use of molecular tests on smear positive respiratory samples to rapidly identify the presence of mycobacteria and screen for rifampicin drug resistance in *M.tuberculosis* complex.
- Molecular tests on smear negative samples and smear positive non-respiratory samples.
- Molecular tests to screen for isoniazid, fluoroquinolones, amikacin, capreomycin, kanamycin and ethionamide drug resistance in isolates of *M. tuberculosis* complex.
- Culture of smear negative samples.
- Identification of slow growing mycobacterial species from environmental water and air samples e.g. from heater-cooler units and environments where these units are in operation.

3. Services available by contract between requesting service and NHS Lothian

- Processing of routine samples including blood cultures from patients for mycobacteria investigation.

Please note SMRL no longer offers PCR for MTBC detection in formalin-fixed, paraffin embedded tissue. Samples for 16S rDNA PCR may be sent directly by the user to a suitably accredited laboratory such as:

Microbiology, Virology and Infection Control Level 4,
Camelia Botnar Laboratories Great Ormond Street Hospital for Children
Great Ormond Street London
WC1N 3JH
DX number: DX6640203
DX Exchange: Bloomsbury 91WC
https://www.gosh.nhs.uk/wards-and-departments/departments/laboratory-medicine/laboratory-database/16s-pcr/
For histological samples requests a minimum of 4 rolled sections (10µm thick). More preferred if possible to allow repeat extractions.

Department of Microbiology
Leeds General Infirmary
Old Medical School
Thoresby Place
Great George Street
Leeds
LS1 3EX
DX number: DX6281504
DX Exchange: Leeds 90LS
https://www.leedsth.nhs.uk/services/pathology/tests/mycobacterial-non-tb-and-tb-pcr/
For histological samples requests 10 sections 10µm thick

Samples for Mycobacterial Investigations

1. Microscopy
All samples except blood, swabs and urine are routinely examined for direct AFB microscopy (provided there is sufficient specimen).

2. Culture
Our current practice is to inoculate all specimens into liquid and solid media.

3. Identification of Mycobacterial species
All first and second isolates will be identified to species level (or group/complex level where appropriate). Full speciation will occur thereafter at 6 monthly intervals for *M tuberculosis* complex and 12 monthly for NTM. Additional isolates will be assessed phenotypically to ensure they are conducive with this species identification. Additional isolates may undergo full speciation based on clinical need, requirement for sensitivity testing, or where phenotypic assessment does not align with species identified in previous isolates.

4. Molecular tests
Pulmonary specimens from NHS Lothian sources fulfilling NICE based criteria will be tested by a rapid molecular test for MTB complex and rifampicin resistance if sufficient sample is received (2ml minimum volume required). In addition, any AFB smear positive samples are also tested. Rapid molecular testing of both pulmonary specimens and positive cultures is also available for MTB complex and isoniazid, fluoroquinolone, amikacin, kanamycin, capreomycin and ethionamide resistance where clinically indicated.

5. Whole Genome Sequencing (WGS)
The SMRL is currently using WGS for species identification of MTB complex, resistance prediction of MTB complex and genetic relatedness of MTB complex isolates from positive cultures. WGS may also be used in place of, or in addition to MALDI-TOF MS (see below) for identification of NTM in certain circumstances.

Rapid confirmation of MTB complex is achieved initially using lateral-flow immunochromatographic assay or Molecular testing. Phenotypic susceptibility testing may be used to confirm MTB complex susceptibility pattern results if drug resistance is suspected.

6. MALDI-TOF Mass Spectrometry for identification of non-tuberculous mycobacteria

SMRL now utilises MALDI-TOF MS for identification of suspected Non-tuberculous Mycobacteria. Decisions regarding testing modality are based on morphological assessment (ZN microscopy), molecular testing and immunochromatographic assay.

7. Drug susceptibility testing

Susceptibilities are routinely performed on the first MTB complex isolate from each patient and then only repeated at 6 month intervals.

NTM susceptibility testing is based on the ATS/IDSA criteria for diagnosing NTM infection, modified by local experience. NTM susceptibility testing is repeated at 12 monthly intervals. Testing will be performed on:

- all NTM from sterile sites (except for *Mycobacterium marinum* in skin);
- first isolates of *Mycobacterium abscessus* complex, *Mycobacterium xenopi, Mycobacterium malmoense and Mycobacterium kansasii*. Experience suggests that these are likely to be associated with clinically significant disease
- patients with cystic fibrosis, HIV or with significant T cell immunosuppression (if we are informed of the clinical features)

For all other pulmonary isolates of NTM we will not do sensitivity testing unless we are informed that the patient meets ATS criteria for NTM lung disease (please indicate which criteria are met) AND there is a concern about resistance following macrolide exposure. **For isolates from sterile sites such as blood and tissue we will automatically perform sensitivity testing** but not **from** non sterile sites such as urine or faeces unless there is a strong clinical suspicion of infection. To arrange sensitivity testing please email us at:
Loth.Smrl@nhs.scot

The current BTS NTM Pulmonary Disease advice, including comment on ATS/IDSA criteria is at: https://www.brit-thoracic.org.uk/quality-improvement/guidelines/ntm (accessed September 2024).

8. Long term storage of Mycobacterial Isolates

All isolates of *M. tuberculosis* complex will be stored as part of the Scottish repository for mycobacterial cultures. For NTMs, only isolates which have undergone full speciation will be stored. Isolates of *M. gordonae* will not be stored long term as these are considered environmental contaminants.

9. Additional Information

There may occasionally be situations where cultures are referred for alternative modes of testing at one of the UKHSA National Mycobacterium Reference Service laboratories.

Users should inform us in advance of cultures that are being sent by post or courier preferably by 'generic' e-mail: Loth.Smrl@nhs.scot. We will issue a receipt report within one working day from receipt of cultures.

For urgent requests or for direct molecular detection add-on testing, a SMRL staff member should be contacted by phone (see contact list above).

A completed SMRL request form **must** accompany every culture or specimen.
https://edinburghlabmed.co.uk/Specialities/reflab/Mycobacteria/Pages/default

Wherever possible, please time the posting of samples and cultures to minimise the possibility of these lying unattended over weekends and holidays. The SMRL will attempt to process all primary samples and referred cultures as much as possible. All required information on the sample or request form that is missing could result in a delay in sample processing.

All requests must provide the following Minimum Data Set information:

Forms

1. Patient Identifier Number (CHI-for Scottish patients)
2. Postcode of patient where available
3. Sender's Laboratory Number
4. Surname
5. Forename
6. Date of Birth
7. Gender
8. Location of Sender
9. Specimen type (or, if culture isolate, specimen type from which the culture was isolated).
10. Any relevant clinical information. If results need to be telephoned please supply contact details.
11. AFB and molecular results were performed at sending laboratory
12. If possible patient's hospital and consultant.

Sample

Minimum data are also required on the sample container:

1. Surname
2. Forename
3. Date of Birth
4. Sample date and time
5. Sample type and site
6. Location of patient
7. Sending Laboratory number if applicable

Vials

On culture vials, patient name and the sender's Laboratory Number are required as a minimum.

Specimens should be correctly collected and delivered as quickly as possible to the laboratory. Although *Mycobacteria tuberculosis* may survive in sputum for one week, the probability of successfully culturing AFB bacilli decreases with time and is especially critical for paucibacillary

specimens. If specimens cannot be transported to the laboratory within one hour, it is recommended to store them at 4 °C. This does not apply to whole blood or bone marrow specimens in Myco/F Lytic bottles which should not be refrigerated or incubated prior to sending. Leaking cultures will not be processed.

10. Interferon Gamma Release Assay (IGRA) for detection of latent TB infection. Not appropriate for active TB infection.

Testing is not a reference laboratory test and is currently performed 'in-house' for NHS Lothian patients by Blood Sciences Autocore using the QuantiFERON-TB Gold Plus (QFT-Plus) test. Other Health Boards may use this facility via a Service Level Agreement with NHS Lothian. Potential users should contact:

Mrs Nadine Wilkinson
Tel: 0131 672 0103
nadine.wilkinson@nhs.scot
Healthcare Science Manager for Blood Science and POCT

Quantiferon 4 tube system and Sarstedt universal blood culture adaptors are available from catalogue on PECOS system for NHS Lothian users. If a Safety-Multifly is used, the line must be primed with an empty Sarstedt tube first to ensure the correct volume (1ml) of blood is delivered into each tube. All 4 tubes (Nil, TB1, TB2, and Mitogen) must be inoculated. The black mark on the side of the tubes indicates the validated range of 0.8–1.2 ml. If the level of blood in any tube is outside the range of the indicator mark, a new blood sample should be obtained. It is important to gently agitate the tubes ten times to ensure the entire inner surface of each tube is coated with blood, to dissolve antigens on tube walls. DO NOT shake vigorously.

Samples should be dispatched to the laboratory as soon as possible but must reach the laboratory within 16 hours of collection. Do not refrigerate. See the Guidelines for NHS Lothian users of Microbiological and Virological Specialist laboratory for full details. Laboratories out with NHS Lothian may incubate tubes overnight at 37ºC which may then be sent according to SLA to the NHS Lothian Specialist Serology/Virology centre.

The T-spot assay is another IGRA, which is available from:

Oxford Immunotec
115D Milton Park
Abingdon
Oxfordshire
OX14 4RZ
Tel: +44 (0) 1235 442 780
Fax: +44 (0) 1235 442 781
www.oxfordimmunotec.com

Note that tuberculin skin testing is an alternative mode of assessing latent TB infection (LTBI). There is no gold standard for LTBI detection. All tests may be negative in patients with active TB infection and LTBI tests have little role in routine assessment of active TB infection.

Sample Types and Containers for Mycobacteria Investigation

Specimen type	Containers and transport	Comments
Sputum (3 to preferably 5ml).	Disposable plastic, sterile "universal" container. Collected approximately 8-24 hours apart. Early morning samples preferred before ingestion of food	Decontaminated and neutralised samples may lose viability during transit and should not be sent
Induced sputum	Disposable plastic, sterile "universal" container	
Bronchoalveolar lavage (BAL)/ Bronchial Washings (3-25ml)	Disposable plastic, sterile "universal" container	
Pleural fluids & aspirates (up to 25ml)	Disposable plastic, sterile "universal" container	Pleural and pericardial fluids are insensitive samples. Concurrent pleural or pericardial biopsy taken with the fluid is more useful
Biopsy material (including pleural biopsies)	Disposable plastic, sterile "universal" container (add a few drops of preservative free 0.9% sterile saline to keep sample moist)	DO NOT USE FORMAL SALINE OR FORMALIN
Tissues (including heart valves)	Disposable plastic, sterile "universal" container (add a few drops of preservative free 0.9% sterile saline to keep sample moist)	DO NOT USE FORMOL SALINE OR FORMALIN
Early Morning Urine	Sterile 500ml bottles filled up to 2/3 full. Send three early morning samples on successive days. Do not use bottles with boric acid. Midstream EMU is an acceptable but not ideal alternative.	EMU bottles available on request from the stores department, Astley Ainsley Hospital, Edinburgh for Lothian users.
Blood (1-5ml, optimum 3-5ml))	Myco/ F lytic blood culture bottles available from SMRL. Citrated blood also acceptable alternative at present.	Do not refrigerate or pre-incubate inoculated bottles.

Bone marrow	Use Myco/ F lytic blood culture bottles available from SMRL, citrated blood or disposable plastic, sterile "universal" container. The latter if AFB smear is required.	Do not refrigerate or pre-incubate inoculated bottles. Skin preparation is key to reducing contamination.
CSF	Disposable plastic, sterile "universal" container	Recommended minimum volume of 6mls. Smear and culture of 6mls CSF may have greater sensitivity than PCR.
Stools **(not recommended)** (minimum 1g)	Disposable plastic, sterile "universal" container with spoon	**These are rarely informative and are heavily contaminated. Not recommended.**
Gastric Washings	Disposable plastic, sterile "universal" container	Acidity of gastric contents can affect Mycobacterial viability. Samples should be transported to laboratory directly, or neutralisation with sodium bicarbonate solution should be considered.
PM specimens	Disposable plastic, sterile "universal" container or larger screw capped sterile container if appropriate	DO NOT USE FORMAL SALINE OR FORMALIN
Pus	Disposable plastic, sterile "universal" container Pus swab may be acceptable. Use 'Stuart's' transport media for pus swabs.	Pus is always preferred if available. Microscopy is not routinely performed on swabs.
Cultures	Egg medium slopes or aliquots of liquid medium	At least 5mls required if grown in liquid medium
Water and air from heater-cooler units (50 ml)	Sodium thiosulphate should added to the water collection pots to neutralise the hypochlorite before water sampling (final concentration of at least 18mg/L)	Air sampling plates supplied by SMRL by prior arrangement.

Tests Available, Turn Around Times and Costs

Turnaround times for cultures are dependent on receipt of a pure culture containing sufficient mycobacteria for analysis.

The time interval between receipt of a culture and the issue of the final identification and first line sensitivity report varies greatly from 1-12 weeks, depending on factors such as the nature of the

culture medium used by the sending laboratory, paucity of organisms in the culture, the species of Mycobacteria and the presence or absence of contamination.

Target turnaround times to result availability for 95% of samples:

Test	**Turnaround time in working days) (**5d/ week laboratory service)
Primary sample AP smear microscopy	≤1d from receipt of specimens (mean 0.3d)
Automated liquid and conventional solid culture on all samples being processed for mycobacterial culture	Set up ≤1 working day from receipt of specimens (Mean 1d)
Clinical specimen examination, culture, isolation and identification where the isolate is subsequently shown to be MTB complex	≤42d from receipt of specimens (mean 12.4d)
Provisional identification of MTB complex	≤7d from receipt of a positive cultures (mean 1.2d)
Identification of MTB complex by WGS	≤14d from receipt of a positive cultures (mean 11.5d)
Identification of NTM by MALDI-TOF MS	≤14d from receipt of a positive cultures (mean 8.1d)
Sensitivity testing of MTB complex[#]	≤28d of isolate receipt/ isolation (mean 11.5d)
MTB complex relatedness by WGS	≤14d from receipt of isolate (mean 11.5d)
Rapid detection of MTB complex and detection of rifampicin resistance	≤3d from receipt of specimens (mean 1.9d)

associated mutations on clinical specimens	
Rapid detection of MTB complex and detection of isoniazid, fluoroquinolone, amikacin, kanamycin, capreomycin and ethionamide resistance associated mutations	≤3d from receipt of specimens

First line susceptibility results availability

The costs for NHS users are reviewed annually. These, plus costs for private tests are available on request.

Test	Method	Test frequency and turn around time	Comments
Microscopy for acid-fast bacilli (AFB) detection	Auramine Phenol staining	Within 1 working day if receipted prior to mid-day (Mon-Fri)	All specimens examined by AP microscopy are also cultured for mycobacteria.
Primary Culture	Solid (modified LJ pyruvate egg slope) and rapid liquid culture (MGIT)	Daily. Usually available 2-8 weeks after receipt of sample. Cultures are reported negative at 8 weeks if appropriate but kept for 12 weeks in total.	Results from contaminated specimens may be delayed
Species identification from cultures	Whole Genome Sequencing. MALDI-TOF MS	Weekly (Thurs); 7 days Three times weekly (Tue/Wed/Fri); 7 days	Rapid confirmation of MTB complex is achieved using lateral-flow immunochromatographic testing in conjunction with morphology, or Molecular testing
Drug susceptibility testing of MTB complex	Whole Genome Sequencing.	Weekly (Thurs); 7 days	Some resistance predictions require confirmation by phenotypic testing which may delay some results. Phenotypic Tests for 3rd line TB drugs set up on request
Drug susceptibility testing of non-tuberculous myco “Slow Growers”	Broth microdilution	Set up weekly as required; 7-14 days incubation	For slow growing NTM mycobacteria. We are unable to test susceptibility to clofazamine.
Drug susceptibility	Broth microdilution	Set up weekly as required; 3-14 days incubation	For rapidly growing mycobacteria. We are unable to test susceptibility to clofazamine.

testing of "Rapid Growers"			
Rapid detection of MTB complex and detection of rifampicin resistance associated mutations	real time PCR (Cepheid)	Daily; usually available on day/day after receipt if received prior to mid-day.	Test is only validated for pulmonary samples although may provide useful for other specimen types. Pulmonary samples from NHS Lothian patients requesting TB investigation are routinely tested if they meet NICE based criteria: BAL samples (non ITU); HIV positive/significant immunosuppression; high index of TB suspicion; TB contact/ potential for large scale contact-screening; travel/residence in high incidence and/or MDR-TB country; on specific request by a consultant in respiratory/consultant infectious diseases; <15 years of age. Charged as not a reference laboratory funded test
Rapid detection of MTB complex and detection of isoniazid, fluoroquinolone, amikacin, kanamycin, capreomycin and ethionamide resistance associated mutations	real time PCR (Cepheid)	Daily; usually available on day/day after receipt if received prior to mid-day.	Run by specific request of a consultant in respiratory/infectious diseases where there is a high index of suspicion for isoniazid mono-resistance, multidrug –resistant (MDR) or extensively drug-resistant (XDR) TB. Charged as not a reference laboratory funded test.
M. tuberculosis complex strain typing (MTB Complex relatedness)	WGS	Weekly (Thurs); 7 days	All new TB patients' isolates are sequenced.

Molecular Detection of Mycobacteria in Clinical Specimens

Note on rapid molecular tests: false negatives and false positives can and rarely do occur.

We currently use Xpert MTB/RIF Ultra (Cepheid) for the direct detection of MTBC in clinical specimens and for the rapid detection of rifampicin resistance associated mutations. This is performed on a daily basis for those NHS Lothian pulmonary patient specimens fulfilling NICE based criteria and for any AFB smear positives specimens.

For detection of isoniazid mono-resistance, MDR or XDR TB, we use Xpert MTB/XDR (Cepheid). This test can be run on both primary samples and positive cultures. Please note that this test detects a limited number of common mutations associated with drug resistance. Resistance will not be detected if alternative mutations occur. This test is run by specific justification and request of a consultant in microbiology, respiratory or infectious diseases.

Reporting of Results

Policy and procedures for telephoned results

Previously unknown AFB smear positive or discrepant samples are telephoned as soon as possible, usually within 1 working day of receipt. New positive MTB complex cultures obtained from smear negative samples are also phoned as soon as possible after their presence is confirmed in the culture.

All NTM are reported and/or E-mailed. 'New' *M.abscessus* from NHS Lothian locations are telephoned in addition.

The following examples will also be telephoned or communicated urgently:

- results of direct rapid molecular tests on specimens if findings are unexpected.
- clinically important identification or drug susceptibility test results (e.g. primary resistance to first line drugs; acquired new resistance; unexpected mycobacterial species).
- new positive cultures from sterile sites or precious samples.

Emailing of reports to secure nhs.net or nhs.scot accounts is set up already for all Scottish user laboratories. In these instances no written report is sent out. We can set this up if you have such a secure laboratory address to automatically email each time there is an updated report. Please contact Mori Robertson in the first instance if you wish to use this means of reporting.

Written report formats

Initial Report on specimens

Microscopy results

Acid Fast Bacilli NOT seen

Culture report to follow

Microscopy results

Acid Fast Bacilli seen (enumeration given)

Culture report to follow

Microscopy results

Microscopy findings equivocal. Advise repeat sample.

Equivocal result means 1-2 acid fast bacilli seen per 300 fields. This is NOT a definite positive result and artefacts could cause this level of acid fast material in a specimen.

Culture report to follow

(approximately ½ such results are culture negative)

If rapid molecular testing is also performed on sample:

M. tuberculosis complex PCR

No MTB complex detected in this specimen on direct PCR.

This does NOT rule out MTB complex or non-tuberculous mycobacterial infection.

M. tuberculosis complex PCR

MTB complex DETECTED in this specimen on direct PCR, consistent with active infection but subject to culture and subsequent confirmatory results.

No mutation indicating rifampicin resistance detected. NOTE:- This test is unable to detect true resistance to rifampicin in a small percentage of isolates. This result is to be confirmed by isolation and susceptibility testing of the organism.

M. tuberculosis complex PCR

MTB complex DETECTED in this specimen on direct PCR, consistent with active infection but subject to culture and subsequent confirmatory results.

Mutations indicating resistance to rifampicin DETECTED.

This result is to be confirmed by isolation and susceptibility testing of the organism.

M. tuberculosis complex PCR

MTB complex DETECTED in this specimen on direct PCR, consistent with active infection but subject to culture and subsequent confirmatory results.

Rifampicin resistance probe uninterpretable

M. tuberculosis complex PCR
M.tuberculosis complex probe uninterpretable.

Initial Reports on Cultures received
Confirmation as a Mycobacterium with species identification is normally reported within 14 working days of receipt of a culture. This is achieved by AFB microscopy to confirm the presence of mycobacteria and WGS or MALDI-TOF MS for identification of cultures.

Initial rapid confirmation of MTB complex is achieved earlier using a lateral-flow immunochromatographic assay.

Final Report on Cultures received
Confirmed species identification with first line MTB complex sensitivity results may take from as little as 7 days to up to 6 weeks. This is achieved using WGS with confirmation of resistance predictions performed using phenotypic DST where appropriate.

Confirmed species identification of NTM may take 7-14 days. This is achieved predominantly using MALDI-TOF MS. Phenotypic sensitivity testing if performed, is batched on a weekly basis.

M. tuberculosis complex genotyping results are reported when completed and comparisons of the genotypes of strains suspected to be clustered are reported.

Cumulative reports
Cumulative results on a patient with positive results can be produced on request. Please contact senior BMS or medical staff at SMRL.

At present only Authorised Final reports on each ‘test’ are available on SCI store. Interim results are not available.

Technical, Scientific and Health and Safety Advice

The technical and scientific staff at the SMRL are happy to provide advice on all laboratory aspects of microscopy and isolation of Mycobacteria and on the safe running and design of containment level 3 laboratories.

Please contact BMS Team Managers for technical help and advice.

Clinical, Epidemiological and Infection Control Advice

The primary source of medical advice for the diagnosis, management and public health investigation of TB lies with the consultants in the relevant specialties in the local health board. The consultant microbiologists attached to the SMRL are happy to complement this primary advice where appropriate.

Please contact Dr Ian Laurenson (Director), Dr Olga Moncayo (Deputy Director), Clinical Scientists or BMS Team managers for advice on issues such as microbiological diagnostic tests, antibiotic susceptibility tests, strain typing for contact tracing, and infection control.

Training and Education

Audit Study Day

Every two years the laboratory hosts an Audit/Study day or online Webinar to allow SMRL staff to meet and discuss various issues with reference laboratory users, public health consultants, chest physicians and Health Protection Scotland (HPS) representatives.

Attachments and visits

Each year SMRL provides training attachments for staff from other laboratories including Biomedical Scientists, Clinical Scientists and Resident Medical Staff. SMRL also provides some support for undergraduate science and medical students, MSc and PhD students and projects for Resident Doctors.

Visits and lectures by SMRL staff to other institutions

Staff at SMRL provide teaching and advice at local and national level on invitation particularly in the areas of diagnosis, patient management and molecular epidemiology.

Contact details

To discuss short visits to SMRL for training purposes, please contact BMS Team Managers or Mrs Mori Robertson, Reference Laboratory Administrator in the first instance.

Research and Audit

Staff are willing to collaborate in relevant audit and research activities. This may take the form of simple data searches to more formal grant applications dependent on ethical approval where relevant and availability of resources.

Contact details
Please contact Dr Ian Laurenson (Director), Dr Olga Moncayo (Deputy Director) or Dr Louise Seagar (Clinical Scientist) for discussions regarding research/audit collaboration.

Quality Assurance

The SMRL is committed to high quality laboratory analysis and is accredited by the United Kingdom Accreditation Service to ISO 15189:2012 for the scope of testing. Identification of NTMs by MALDI-TOF MS, susceptibility testing to non-tuberculosis mycobacteria, direct molecular detection of non pulmonary samples and Environmental water sampling is currently out with our scope of accreditation.

The laboratory participates in the UK National External Quality Assurance (NEQAS) scheme for Mycobacteria microscopy, culture and molecular typing as well as Quality Control for Molecular Diagnostics (QCMD) for molecular detection in specimens. The laboratory also participates in the WHO 'Drug Proficiency Testing Scheme', an external QA scheme for sensitivity testing of *M tuberculosis* isolates. The laboratory also participates in the INSTAND Mycobacteria identification and Mycobacteria Drug Susceptibility Testing of Non-tuberculous Mycobacteria EQA program. For typing, the laboratory partakes in the international proficiency study on WGS by the National Institute for Public Health and the Environment TB Reference Laboratory Netherlands (RIVM).

We also carry out Internal Quality Assurance (IQA).

The SMRL User Manual is available at the Scottish Microbiology Reference Laboratories, Edinburgh website
http://www.edinburghlabmed.co.uk/Specialities/reflab/Pages/default.aspx
(accessed December 2024)

Protection of Personal Information

The SMRL and NHS Lothian Mycobacteria Laboratory comply with the Data Protection Act. This requires all organisations which handle personal information to comply with a number of important principles regarding privacy and disclosure.

Antimicrobial Assays

The following information is for assays on first line TB therapy drugs.

Isoniazid, Pyrazinamide and Ethambutol

Provider	Cardiff Toxicology Laboratories
Contact Name	Katie Jones, Clinical Scientist, katie.jones14@wales.nhs.uk
Address For Specimens	Toxicology Laboratory The Academic Centre Llandough Hospital Penarth CF64 2XX
Country	United Kingdom
Tel. No. (Voice)	029 2071 6894
Tel. No. (Fax)	029 2035 0142
E Mail (click to mail)	Katie.Jones14@wales.nhs.uk
Accreditation Status	Accredited
Other Information	For general enquiries please contact Emma.Taylor6@wales.nhs.uk
Web Site (click to view)	http://www.ctlabs.co.uk

Rifampicin, Moxifloxacin and Levofloxacin

Provider	Antimicrobial Reference Laboratory, Southmead Hospital, Bristol
Contact Name	Alan Noel
Address For Specimens	Department of Medical Microbiology Southmead Hospital Westbury on Trym Bristol BS10 5NB
Country	United Kingdom
Tel. No. (Voice)	0117 4146220
Tel. No. (Fax)	0117 4146282
E Mail (click to mail)	arlenquiries@nbt.nhs.uk
Accreditation Status	Accredited
Other Information	
Web Site (click to view)	Requesting | North Bristol NHS Trust (nbt.nhs.uk)

Leprosy Diagnosis

The SMRL does not undertake investigations for the diagnosis for leprosy.
UK wide information and advice can be found at:
Memorandum on leprosy 2023 - GOV.UK

Clinicians should refer all suspected or confirmed cases to one of the national leprosy centres where the case can be reviewed by a Consultant Advisor in Leprosy in London, Liverpool or Birmingham. For the HDT London contact in the first instance:

Dr. Stephen Walker
Consultant Advisor in Leprosy
Hospital for Tropical Diseases
Mortimer Market Centre
Capper Street
London
WC1E 6JB
Tel: 020 3447 5959
uclh.htdadmin@nhs.net

Detection of *M. leprae* by PCR is available at Great Ormond Street Hospital. Samples should be sent directly by the user to:

Microbiology, Virology and Infection Control Level 4,
Camelia Botnar Laboratories
Great Ormond Street Hospital for Children
Great Ormond Street
London
WC1N 3JH
DX number: DX6640203
DX Exchange: Bloomsbury 91WC