Irish Mycobacteria Reference Laboratory

(IMRL)

St James's Hospital

User Manual

The Irish Mycobacteria Reference Laboratory (IMRL)

The Irish Mycobacteria Reference Laboratory is the Irish Reference Laboratory for Mycobacteriology. Established in 2001, the laboratory performs a specimen and culture referral service for hospitals throughout Ireland. Approximately 6,000 specimens are processed annually and the laboratory receives almost 500 mycobacterial cultures per year. The Irish Mycobacteria Reference Laboratory is located in the LabMed Directorate (SJH Centre for Laboratory Medicine & Molecular Pathology) of St. James's Hospital and is fully accredited to ISO 15189 by the Irish National Accreditation Board (Registration Number 327MT).

1 IMRL Services

Services offered by the laboratory include:

- Microscopy and culture of routine clinical specimens for mycobacteria
- Use of molecular tests on smear positive specimens to rapidly identify the presence of mycobacteria and screen for drug resistance in *M. tuberculosis* complex (MTBC)
- Rapid molecular detection of *M. tuberculosis* complex and rifampicin resistance determination performed on smear negative specimens upon request by Consultant Microbiologist
- Species identification of mycobacteria using molecular techniques
- Susceptibility testing of *M. tuberculosis* complex to first line anti -TB agents in addition to WHO-defined Group A, B and C anti- TB drugs (streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide, moxifloxacin, amikacin, kanamycin, delamanid, bedaquiline, clofazimine, levofloxacin, linezolid and pretomanid).
- Identification of MTBC to species level (i.e. *M. tuberculosis, M. bovis, M. bovis BCG, M. africanum* etc) and detection and confirmation of resistance from cultured isolates using whole genome sequencing (WGS) drug resistance prediction
- Assistance with isolation of mycobacteria in difficult cases
- Molecular genotyping and comparison of *M. tuberculosis* complex strains using WGS to augment Public Health investigation of clusters and/or outbreaks.

- Advice to clinicians and laboratories in relation to the diagnosis, treatment and infection control of tuberculosis.
- Referral of resistant *M. tuberculosis* complex isolates for additional drug susceptibility testing
- Referral of NTM isolates for drug susceptibility testing following isolate identification
- Molecular test using PCR and Sanger sequencing for the diagnosis of *M. leprae* (research only test).

2 Request Forms

2.1 Specimen Request Form

Specimens sent to the IMRL must originate from the Microbiology/Pathology departments in the client hospital. Hospitals/Users should use the external form LF-IMRL-0195 downloadable from the SJH website (IMRL User manual). Laboratory numbers should be assigned to the forms and specimens before they are forwarded to the IMRL. This number will be quoted on all future correspondence from the IMRL.

The request form LF-IMRL-0195, in legible writing, must accompany specimens and it **must** contain this minimum set of information:

- Hospital name
- Full name
- Patient's address
- Medical Record Number (MRN)
- Date of Birth (DOB)
- Sex
- Site of infection
- Clinical details (Essential if Non-tuberculous Mycobacteria, NTM, are suspected)

If minimum criteria are NOT provided, specimens will be rejected.

The consultant microbiologist in the client laboratory will be, unless otherwise agreed, the name on the IMRL report.

Microbiology will be, unless otherwise agreed, the "ward" name on the IMRL report.

The IMRL Specimen Request Form can be downloaded from the IMRL website at (http://www.stjames.ie/Departments/DepartmentsA-Z/I/IMRL/DepartmentOverview/)

IMRL Specimen Request Form

2.2 Positive Culture Request Form

Isolates/Cultures that are positive for AFB originate from the Microbiology departments in the client hospital. Laboratory numbers should be assigned to the cultures before they are forwarded to the IMRL. This number will be quoted on all future correspondence from the IMRL.

The request form should be filled out in legible writing and must contain this **minimum** set of information.

- Hospital name
- Full Patient name
- Patient's address (mandatory for notification of TB)
- Medical Record Number (MRN)
- Date of Birth
- Sex

If minimum criteria are NOT provided, specimens will be rejected.

The IMRL Culture Request Form can be downloaded from the IMRL website at (http://www.stjames.ie/Departments/DepartmentsA-Z/I/IMRL/DepartmentOverview/)

- IMRL Positive Culture Request Form

3 Labelling of Specimens/ Cultures

3.1 Specimens

The specimen must contain at least two patient identifiers, that is, Name plus DOB or MRN, in order to be processed. Specimens **not containing** at least 2 patient identifiers will be rejected.

3.2 Isolates/Cultures

All cultures must be labelled with the following information:

- Full name and at least one of the following: MRN, DOB or External laboratory number

4 Specimens for Mycobacterial Investigations

4.1 Nucleic Acid Amplification Tests (Direct)

To be performed to a sufficiently high standard, NAA tests require the proper molecular facilities to be available in addition to the appropriate expertise and experience to interpret results. These tests are neither 100% sensitive nor 100% specific. The appropriate number of specimens to test with NAA will vary depending on the clinical situation, the prevalence of TB, the prevalence of NTM and laboratory proficiency. Specific algorithms are available and need to be employed for proper interpretation of results. While there are publications regarding the use of NAA methodologies on non-respiratory specimens, caution is still required. It is generally recommended that these tests be carried out in reference facilities. With a worldwide increase in MDR-TB, amplification tests have a potentially important role to play in containment of resistant disease. The British Thoracic Society provides guidelines on when these tests should be considered. GeneXpert MTB/RIF Ultra and MTB/XDR assays are NAATs available at the IMRL for identification of MTBC and drug resistance prediction directly on specimens (and positive cultures in the case of MTB/XDR), see section 6.1.1.
Note: No NAAT is 100% sensitive or specific. Molecular markers 'not detected' cannot completely outrule mycobacterial infection or the presence of drug resistance. Similarly, novel mutations could lead to false detection of resistance.
The following requirements **must be met** when a NAAT test is requested from an external source (eg. laboratory and/or clinical staff):

4.2 Specimen Requirements:

Sputum/ BAL: minimum volume of 1 ml (decontaminated, or un-decontaminated)

Gastric washings: minimum volume of 1 ml

CSF, body fluids, **eg. pleural fluid**: preferably 0.5 ml should be received. If lower volumes are received, this will greatly affect the sensitivity of the assay.

Blood: minimum volume of 1 ml

Lymph node and tissue specimens, bone marrow, abscess contents, aspirated pus: as much specimen as possible

Urine: minimum volume of 1 ml

4.3 Specimens for mycobacterial (TB) culture:

Successful isolation of mycobacteria is greatly affected by delays between specimen procurement and specimen processing in the laboratory. This is especially so if the specimen is from a non-sterile site, e.g. sputa, bronchoscopy specimens and urine. Consequently, when a specimen is procured it must be sent to the laboratory immediately. Batching of specimens is not recommended. Should a delay be unavoidable (e.g. weekends), specimens should be refrigerated until transported to the IMRL. Specimens must be obtained in a manner that has due regard to the safety of the staff who will handle them when they arrive in the laboratory. This implies that containers should be robust, checked for leaks and not contaminated on the outer surfaces. Forms and specimens should not be transported in the same bag.
Transportation of specimens to the laboratory must occur in a safe manner and comply with the appropriate regulations (Packaging Instruction P650).
Guidelines given below are for those institutions that the IMRL has agreed to provide a culture service for.

4.3.1 Acceptable Specimens

The following specimens are acceptable for culture.

- Sputum
- Specimens obtained at Bronchoscopy
- Aspirated fluids and pus
- Tissue
- Gastric aspiration
- Blood
- Bone marrow
- CSF
- Urine in certain circumstances (See Below)

4.3.2 Unacceptable Specimens

- Poor quality sputum specimen's e.g. salivary specimens or specimens of minute quantities.
- Faeces
- Tissue in fixative
- Urine, except when the following is **stated** on the request form:

- A diagnosis of renal or miliary tuberculosis is suspected.
- Relevant clinical details are provided, e.g. “Sterile pyuria” “Haematuria”
- The patient is immunocompromised.
- The patient is under the care of a Nephrologist or Urologist
- Following prior discussion with the laboratory director

If a urine specimen is received **without mention of one of the above categories,** it will not be processed so we ask you to endeavour to have the form properly filled out. An early morning MSU or CSU specimen, taken into a sterile plastic container, should be procured and immediately submitted to the IMRL on each of three successive days.

4.3.3 Sputum

The specimen should be:

- Taken before the commencement of therapy
- Collected safely: appropriate container with wide mouth to avoid contaminating outside
- Coughed from deep in the lungs, not saliva
- An early morning specimen
- Procured and submitted on each of three consecutive days

It should be noted that:

- Three specimens yield >95% recovery but they should not be pooled.
- The patient should be instructed how to take specimen.
- Specimens should be taken in a dedicated room to avoid possible transmission of infectious agents
- A good specimen should be between 2 and 5 ml.
- The patient should not clean teeth or use antiseptic mouthwash before specimen procurement.

4.3.4 Bronchoscopy and other Aspirated Fluids

Specimens should be taken into in sterile screw-capped containers without any additives. Caps should be tightened firmly and the containers checked to ensure they are not leaking. Specimens should be sent to the IMRL on the day they are procured. Store the specimen at 4^{o}C if a delay is unavoidable.

4.3.5 Pus

As much pus as is possible should be collected into a plastic sterile container and the screw cap tightened firmly. Swabs dipped in pus are rarely satisfactory and should be regarded as a last resort. Specimens should be sent to the IMRL on the day they are procured.

4.3.6 Tissue

Tissue is preferable to necrotic material or pus as the latter contain free fatty acids that are toxic to *Mycobacteria sp*. Sterile plastic universal containers **without** additives/ fixatives are suitable for the specimen. If the specimen is unlikely to reach the IMRL within 24 hours, a volume of sterile saline sufficient to cover the tissue should be added to the container. Most histological fixing solutions are toxic to *Mycobacteria sp*. Specimens procured at post mortem should be transferred **immediately** into the container to avoid potential exposure to Formalin vapour at the time of autopsy. Specimens should be sent to the IMRL on the day they are procured.

4.3.7 Blood

Blood for mycobacterial culture should be inoculated directly into a BACTEC MycoF/Lytic blood culture bottle according to the manufacturer's instructions. The range of blood volume which can be cultured is 1 mL to 5 mL, with optimum recovery obtained at 3 mL to 5 mL. Limitations of the procedure outlined in the manufacturer's instructions state:

*"**BD BACTEC** Myco/F Lytic vials **are not selective** and will support the growth of other aerobic organisms besides mycobacteria, yeast and fungi. Positive vials may contain one or more species of mycobacteria and/or other non-mycobacterial species. If present, **fast growing organisms may mask the detection of slower growing mycobacteria, yeast and fungi.** Subculture and additional procedures are required. The consistency of microscopic morphology in BD BACTEC Myco/F Lytic has not been established. **False positivity** most likely will increase when the **blood volume is above 5 mL. False negative readings** may result when certain organisms are present which do not deplete O2 levels sufficiently to allow detection by the system.*

As BACTEC MycoF/Lytic blood culture bottles are now made of plastic, the pneumatic tube transport (PTT) system can be used for receipt and dispatch of these plastic vials. For external hospitals, specimens should be sent and addressed to the Microbiology Department (SJH) on the day they are procured. The microbiology department in the client hospital should supply the culture bottles to the ward. An external microbiology laboratory can request the BACTEC MycoF/Lytic blood culture bottles from the IMRL following an appropriate request. Because of the short expiry date on the bottles, a limited supply is issued for use.

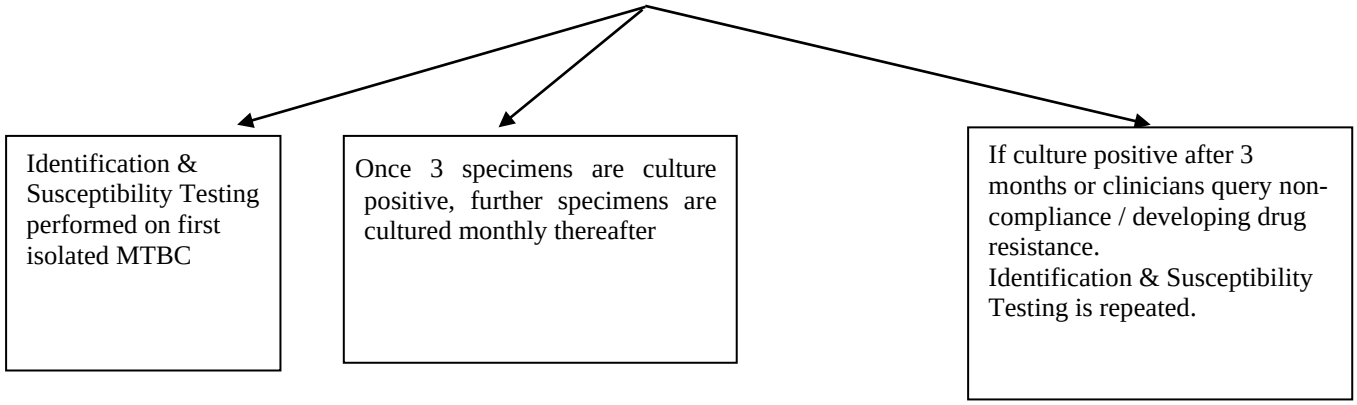
4.3.8 Bone Marrow

The volume of bone marrow obtained determines how the specimen should be collected. Specimens of less than 1.0 ml should be taken into a plastic sterile universal container. Specimens of greater than 1.0 ml should be inoculated directly into a BACTEC MycoF/Lytic blood culture bottle. Specimens should be forwarded to the Microbiology Department (SJH) immediately. If microscopy is required, the smears should be prepared when the specimen is obtained and sent along with the culture material.

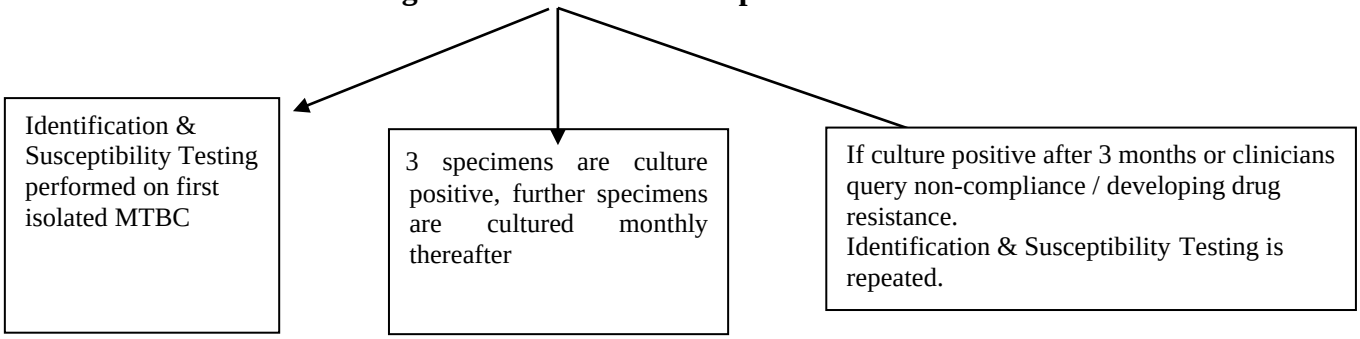
4.4 Protocol for Processing Multiple Positive Specimens from the same Patient

Occasionally the Irish Mycobacteria Reference Laboratory (IMRL) receives multiple specimens from a patient who is smear and/or culture positive. This protocol outlines the IMRL policy to deal with such situations. Processing more than 3 microscopy positive specimens from patients can lead to an increase in the incidences of cross contamination in a laboratory.

Smear Positive Specimens of *Mycobacterium tuberculosis* complex (MTBC)



Smear Negative Culture Positive Specimens of MTBC



Note;

ID/SENS is performed on one isolate only. In our experience, isolates from differing body sites within the same episode (<3 months) tend to have the same genotype and susceptibility pattern. Following an audit, isolates from differing body sites will only be genotyped on special request. Following genotyping, if a different genotype is detected at that point, a full sensitivity profile will be performed on that isolate.

If a patient remains culture positive following **3 months** treatment or if clinicians query **non-compliance or developing resistance**, identification and susceptibility testing is performed.

4.5 Isolate/Culture Requirements

In order to minimise turnaround-times we are requesting that a minimum of 2 vials/Eppendorf tubes each containing 1-2 mls of positive liquid culture are referred for testing. In order to ensure safe handling of positive cultures in the IMRL, please use vials/Eppendorf tubes with screw cap lids ONLY. Alternatively, an LJ slope in a plastic container, or a Middlebrook medium agar plate sealed with parafilm may be sent for examination.
Only pure cultures of mycobacteria will be processed at the IMRL.
Contaminated cultures will not be processed.

Liquid and solid cultures should be transferred into a sterile plastic container (x 2) and the lid sealed with Para-film. Due to safety considerations LJ slopes received in thin-walled glass containers will **not** be processed (in this case subculture from the LJ slope into an Eppendorf tube containing Middlebrook 7H9 broth). In the CAMLIC systems there tends to be large amounts of bacilli present when vials become culture positive. These tend to clump at the bottom of the tube or vial. If this "sediment" is aspirated and transferred into a sterile plastic container, rapid molecular testing can be performed directly on the aliquot received, with the result that we seldom have to wait until a subculture has grown to perform identification tests, decreasing turnaround times.

5 Packing and Transporting of Infectious Substances

5.1 Packing and Transporting of Specimens

Regulations require specimens to be packaged under U.N. guidelines and transported using a courier service. Specimens of any kind cannot be sent by post. A brief description of the requirements for both packaging and dispatch are outlined below, for the complete version see Appendix 1.

- When a specimen is to be dispatched to any laboratory inside or outside of Ireland, it must be in a suitable container. These include micro-tubes, bijou bottles and universal containers. Parafilm should be used to seal the tops of the containers.
- Each container should be in a sealed plastic bag which or may not have a form attached.

- All the specimens can then be placed into a single secondary container, usually plastic, which is then placed in a tertiary container.
- The forms accompanying the specimens, if there are not attached to the specimens, can be placed in a single plastic bag and put in the secondary container.

The tertiary container should bear:

Sender's details

U.N 3373 label stating that the box contains "Diagnostic specimens"

24hr. emergency name and contact phone number of the sender.

Destination address

Any other information required by the courier service

PLEASE NOTE: BACTEC MycoF/Lytic blood culture bottles are made of plastic and should be packaged in a suitable container/ package. This package should be marked as "URGENT" and addressed ONLY to the Microbiology Department, SJH.

5.2 Packing and Transporting of Isolates/Cultures

Changes in the ADR regulations 2009 allow for the transport of MTBC by road, under the category UN3373, "Biological Substance, Category B"[1]. Packing instructions P650 apply.

- Each container (primary container) should be wrapped in enough absorbent material to contain the contents, should it leak. This should be placed in a sealed bag. At the very minimum it should be placed in a plain plastic bag folded over.
- This bag should be placed in a plastic screw topped container (secondary container) that is then fitted into a cardboard box (tertiary container).
- The Request form (in a separate plastic bag), accompanying the specimen should also be placed in the secondary container.
- Labels with the following information should be applied to the cardboard box:
    1. Sender's details
    2. 24hr. emergency name and contact phone number
    3. U.N 3373 label, "Biological substance Category B."
    4. Destination address to read as follows:

Irish Mycobacteria Reference Laboratory
SJH Centre for Laboratory Medicine & Molecular Pathology
St. James's Hospital
Dublin 8

Only to be opened in a CL3 facility

Irish Mycobacteria Reference Laboratory

Please also attach the following labels to the outside of the cardboard box;

(See Appendix 2 for IMRL Labels for Transport)

DO NOT OPEN THIS BOX

ONLY TO BE OPENED IN THE

TB LAB IN ST. JAMES'S HOSPITAL

For the Attention of the I.M.R.L.
St. James' Hospital.

ONLY TO BE OPENED IN A CL3 FACILITY
DO NOT OPEN THIS BOX

5.3 Culture Dispatch

Once the cultures have been packaged correctly, send them to the IMRL by courier.

A fax form for culture dispatch must be completed and faxed to the IMRL immediately following dispatch (01) 4103473. Alternatively, email the fax form immediately following dispatch to imrl@stjames.ie.

The IMRL fax and email forms can be downloaded from the IMRL website at (Irish Mycobacteria Reference Laboratory | St James's Hospital)

- Fax form for Culture dispatch

6 Tests Available and Turnaround Times

6.1 Nucleic Acid Amplification Tests (Directly on specimens)

While molecular assays have the ability to detect MTBC DNA in a timely manner, a positive test result does not necessarily indicate the presence of viable organisms; therefore, culture still remains the gold standard method for diagnosing active TB infection.

6.1.1 GeneXpert Assays

GeneXpert MTB/RIF Ultra assay: Rapid detection of MTBC and the detection of rifampicin resistance associated mutations can be performed directly on clinical specimens. This assay can be performed daily Monday-Friday.
Please contact the IMRL before dispatching the specimen for GeneXpert testing.
Requests for GeneXpert testing on smear negative specimens must be made through a Consultant Microbiologist in the SJH Microbiology Department.

GeneXpert MTB/XDR assay (reflex test, can be performed on specimens that are MTB/RIF Ultra positive, and on positive cultures if required): Rapid detection of MTBC and the detection of isoniazid, fluoroquinolone, amikacin, kanamycin, capreomycin and ethionamide resistance associated mutations. This assay is available on request.

GeneXpert MTB/RIF Ultra and MTB/XDR assays cannot detect ALL drug resistance conferring mutations that could potentially be present within a specimen or positive culture.
Note: No molecular test is 100% sensitive or specific. Molecular markers ‘not detected’ cannot completely outrule mycobacterial infection or the presence of drug resistance.

6.1.2 Deeplex Myc-TB assay

The Deeplex Myc-TB assay is a targeted next-generation sequencing assay (recommended by the WHO) that enables identification and detection of drug resistant mutations in *M. tuberculosis* complex from heat-inactivated clinical specimens and/or isolates. This assay covers resistance to first- and second-line TB drugs including rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin, fluoroquinolones, ethionamide, kanamycin, amikacin, capreomycin. In addition, this assay can detect resistant mutations associated with new and repurposed drugs including bedaquiline, clofazimine and linezolid. This assay is available on request in the IMRL.

If a resistance-associated mutation is detected using the Deeplex Myc-TB assay, a comment will be included to report the drug-resistance detected in the sample. A separate report can be produced on special request for MDR/XDR-TB samples if required.

Deeplex Myc-TB assay cannot detect all drug resistance conferring mutations that could potentially be present within a specimen or isolate. No mutations detected does not exclude the possibility of resistance.

6.2 Specimen Referral Service

It is important to note that processing of specimens begins at 8.00am each day. If a specimen arrives after this time it will not be processed until 8.00am the following working day. It is therefore worthwhile that client laboratories send their specimens to the IMRL as soon as possible. Specimens received out of normal hours will be refrigerated on arrival. For example, if specimens arriving in the client laboratory are received and dispatched to the IMRL by 3.00pm Friday or Saturday morning, microscopy results will be available on Monday. If these specimens are held in the client laboratory until Monday morning the microscopy result will not be available until Tuesday.

6.2.1 Microscopy

Microscopy results are available within 2 days of receipt of the specimen in the IMRL on any working day.

6.2.2 Mycobacterial Culture (TB culture)

All specimens are incubated in liquid culture media for 6 weeks and on solid media for 8 weeks. Culture negative reports are issued at 8 weeks.

6.3 Culture Referral Service

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.

6.3.1 Identification

Rapid molecular identification procedures can be applied directly to the culture submitted depending on the quality and quantity of the culture. Identification to MTBC level is carried out daily and identification of NTM is performed routinely with TATs ranging from 7-21 days. The presence of contaminating organisms, (e.g. GNB, GPCs) greatly affects the TAT of molecular techniques.

Identification of NTM is performed using sanger sequencing (HSP-65 and 16-23s ITS targets) and by GenoType line probe assays (Bruker-HAIN Diagnostics).

Sub-speciation within the *M. tuberculosis* complex (MTBC) is performed using whole genome sequencing (WGS). The GenoType MTBC (Bruker/HAIN Diagnostics) assay may also be used for differentiation of MTBC. However, with this assay, on rare occasions, there may be a cross reaction between *M. tuberculosis* and *M. canetti*.

M.abscessus complex will be sub-speciated and have genotypic DST performed every 6 months using the GenoType NTM-DR assay (Bruker/HAIN Diagnostics). GenoType NTM-DR assays cannot detect ALL drug resistance conferring mutations that could potentially be present within an isolate.

Genotypic DST may be performed on other NTM species on request (outside the scope of INAB ISO15189 accreditation). Please contact the IMRL for further information.

6.3.2 Susceptibility Tests

Following receipt of cultures, a new inoculum is prepared and when sufficient growth is obtained, (usually 4-5 days), susceptibility tests to the first line anti-tuberculous drugs Isoniazid, Rifampicin, Ethambutol and Pyrazinamide is performed {Streptomycin is also tested}. These tests usually take between 4-13 days to complete but may take up to 21 days.
The following first line anti-TB drugs are tested routinely at the IMRL:
Streptomycin (1.0 μg/ml, 4.0 μg/ml); Isoniazid (0.1 μg/ml, 0.4 μg/ml); Rifampicin (0.5 μg/ml); Ethambutol (5.0 μg/ml); Pyrazinamide (100 μg/ml).

Susceptibility testing to the following 'second line' and 'new and re-purposed' anti TB agents is available on request:
Fluoroquinolones: Moxifloxacin (0.25 μg/ml, 1.0 μg/ml); Levofloxacin (1.0 μg/ml)
Aminoglycosides: Kanamycin (2.5 μg/ml) and Amikacin (1.0 μg/ml).
New and re-purposed drugs: Bedaquiline (1.0 μg/ml); Clofazimine (1.0 μg/ml); Delamanid (0.06 μg/ml); Linezolid (1.0 μg/ml) and Pretomanid (0.5 μg/ml and 2.0 μg/ml).

For isolates that require susceptibility tests beyond the first line agents, an extended TAT is required as a sub-culture of isolates is required to test against the panel of additional anti-TB drugs (moxifloxacin, levofloxacin, linezolid, bedaquiline, clofazimine, delamanid, pretomanid, amikacin, kanamycin) and to refer to the SMRL/Milan supra-reference laboratories for additional anti-TB agents (only if required).

6.3.3 Drug Resistance Prediction using Whole Genome Sequencing (WGS)

Whole genome sequencing is performed in parallel to phenotypic drug susceptibility testing (pDST) in the IMRL. Resistance prediction using WGS is used to confirm pDST results.

If resistance is detected using pDST, and WGS has confirmed the result, a comment indicating that WGS has confirmed the resistance detected in this isolate using pDST.
If no resistance is detected with pDST, there will be no need for a comment.
If WGS resistance prediction pre-dates pDST results, a WGS report can be produced on special request (outside the scope of INAB ISO15189 accreditation).
See appendix 3 for details of IMRL analysis pipeline.

Note: the most up-to-date version of the WHO MTBC Mutation Catalogue is used to predict resistance. No method is 100% specific and sensitive and therefore 'not detected' does not completely outrule the presence of resistance.

6.3.4 Referral of Isolates for Susceptibility Testing

Phenotypic susceptibility testing of NTM is performed in the Scottish Mycobacteria Reference Laboratory (SMRL) by request. There is a charge associated as the IMRL incurs a charge from the SMRL. The turnaround time is a minimum of two weeks. Please contact the IMRL to request susceptibility testing following the identification of the isolate.

M.abscessus complex will be sub-speciated and have genotypic DST performed every 6 months using the GenoType NTM-DR assay. Genotypic DST may be performed on other NTM species on request. Please contact the IMRL for further information.

Phenotypic susceptibility testing of resistant MTBC isolates for selected drug susceptibility tests (such as prothionamide and ethionamide) is performed in the SMRL on special request. There is a minimum turnaround time of three weeks. Please contact the IMRL if further information is required.

6.3.5 Genotyping and Relatedness Analysis of MTBC Isolates using WGS

Molecular genotyping of all MTBC isolates is performed in the IMRL using whole genome sequencing (WGS). The thresholds on which genetic relatedness are based on the molecular clock and mutation rates estimated by the TB research community for *M. tuberculosis* and not any other members of the MTBC. This method is considered sufficient to predict closely related strains of any of the members of the MTBC. These could be strains recently transmitted from human to human, or those that descended from a common ancestor where intermediates have not been detected to date. This genotyping method cannot predict outbreaks alone and is only indicated to augment and focus public health investigations. For each isolate, a genotype will be reported, and an IMRL cluster code will be assigned in any case where a very closely related strain(s) has been found in the IMRL database (ie a possible case of recent transmission). This information will also be reported to the Computerised Infectious Disease Reporting Database (CIDR). Turnaround times for this service range from 2 - 27 days from date of receipt, depending on quality and quantity of DNA. However, as genotyping is performed routinely, urgent analysis can be performed on

request e.g. outbreak situations or possible cases of laboratory cross contamination. In these instances, the turnaround time will be decreased. Requests for this service can be made to Professor Breida Boyle/ Professor Johannes Wagener, Dr. Margaret Fitzgibbon, Dr. Emma Roycroft or to the laboratory directly.

7 Communication of Results

To facilitate the timely reporting of results, the Microbiology/IMRL department has introduced electronic communication of results.

Users with signed agreements in place with the Microbiology/IMRL department will receive electronic communication of results.

Microbiology Medical staff will phone/communicate all **positive** microscopy, GeneXpert, culture and susceptibility test results and record the details in the LIS system. Client laboratories should provide contact details including name(s) and contact details/phone number(s) of staff to which these reports will be phoned. Staff in client laboratories will be asked for their name etc. when a report is being phoned. This is departmental policy and it is suggested that client laboratories should also have a policy in place for receiving phoned results from the IMRL.

Phoning the IMRL for results should be kept to a minimum. One phone call, around 3.30 pm, is usually sufficient to get the microscopy results for each working day, if required.

The sequence of reports for submitted mycobacterial cultures is usually in the following order:

1. Isolate/culture identification.
    a. Identification to MTBC level. Further speciation will follow.
    b. Identification of NTM.
    c. Rapid detection of MTBC and drug resistance genes by molecular methods.
2. Susceptibility tests
    a. For MTBC isolates the susceptibility test results follow the initial identification.
    b. Phenotypic susceptibility testing for NTM is performed by request in the SMRL. The IMRL will issue a final report when received from the SMRL. Final results will be emailed to Users (where agreements are in place). *M.abscessus* complex

will have genotypic DST performed every 6 months using the GenoType NTM-DR assay. Genotypic DST may be performed on other NTM species on request. Please contact the IMRL for further information.

c. Selected drug susceptibility testing (for resistant MTBC isolates where applicable).

3. *M. tuberculosis* complex genotyping

An interim report is issued with the final identification of the organism and susceptibility tests results until molecular genotyping is complete. Genotyping results are considered the final report at the IMRL. Genotyping results are reported electronically through CIDR to individual Public Health departments.

7.1 Microscopy

The First Smear Positive result for a patient will be phoned/communicated by email by Microbiology Medical staff to the contact supplied by the client laboratory. Microbiology medical staff will record the details of this contact. Negative results will not be phoned.

7.2 Culture

The First Culture Positive specimen from a patient will be phoned/ communicated by email to the contact supplied by the client laboratory. Negative results will not be phoned.

7.3 Identification and Susceptibility

The Identification of a patients' First Isolate will be phoned/communicated by email. Susceptibility test results will be phoned/communicated by email. Our medical staff will record details of each contact.

7.4 Hard Copy Reports

Hard copy reports of microscopy, culture, identification and susceptibility will be sent to the requesting laboratory as soon as they are available. An interim report is issued with the final identification of the organism and susceptibility tests results until molecular genotyping is complete. Genotyping results are considered the final report at the IMRL and are reported electronically.

7.5 Reporting of Unacceptable Urine and Sputa Specimens

Urine specimens that do not fall into the approved categories will be documented and reported as **"Specimen not processed for TB culture"** and "**Urine is an inappropriate**

specimen for the diagnosis of pulmonary tuberculosis in an immunocompetent patient. Please send sputum or discuss with laboratory."

Sputum specimens of poor quality, either salivary or of minute proportions, will not be processed.

These specimens will be documented and reported as **"Specimen not processed for TB culture"** and **"Saliva or insufficient sputum received"**

8 Core Hours

The core laboratory hours are 8 am to 8 pm, Monday to Friday, but not weekends, Christmas and New Year holidays.

Cultures are processed throughout the weekend in order to facilitate rapid turnaround of results

8.1 Protocol for "Out of Hours" Service

Please Note: Urgent microscopy "Out of hours" is no longer performed and has been replaced by the GeneXpert MTB/RIF Ultra assay. This test requires the approval of a Consultant Microbiologist in the Microbiology Department of St James's Hospital. Please contact the laboratory at (01) 4162972 or 4162046 or through the SJH switchboard at 4103000 and ask for the medical scientist "On Call" in microbiology on bleep number 194. The medical scientist will provide the phone number of the Consultant on-call. The decision will then be communicated back to the medical scientist by the Consultant.

If approved:

Send the specimen, appropriately packaged, to:

Microbiology Department

SJH Centre for Laboratory Medicine & Molecular Pathology

St James's Hospital

8.2 Time Limits for Requesting Additional Examinations

All isolates are frozen at -80°C therefore additional requests can be processed.

9 Contact Details

Name and Designation	Telephone Numbers & E-mail Address
Professor Breida Boyle Professor Johannes Wagener Clinical Directors	(01) 4162971 (bboyle@stjames.ie) (01) 8964551 (jwagener@stjames.ie)
Dr Margaret Fitzgibbon Chief Medical Scientist	(01) 4162963 mfitzgibbon@stjames.ie
Dr Emma Roycroft Specialist Medical Scientist	(01) 4151951 eroycroft@stjames.ie
Ms Maeve Keane Senior Medical Scientist	(01) 4284211 mkeane@stjames.ie
IMRL direct phone line	(01) 4284211, 4162980 imrl@stjames.ie

Advice on Clinical, Interpretation and Treatment matters can be obtained from

Professor Breida Boyle/ Professor Johannes Wagener

Professor Joseph Keane (01 410 3920)

Professor Anne Marie McLaughlin (01 410 3920)

Advice on scientific matters can be obtained from

Dr Margaret Fitzgibbon

Dr Emma Roycroft

Ms Lorraine Montgomery

Ms Maeve Keane

Postal Address:

Irish Mycobacteria Reference Laboratory,

Microbiology Department,

LabMed Directorate,

St. James's Hospital,

Dublin 8

10 Packaging Instruction P650 (Appendix 1)

This packing instruction applies to UN No. 3373.

(1) The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during carriage, including transshipment between vehicles or containers and between vehicles or containers and warehouses as well as any removal from a pallet or over pack for subsequent manual or mechanical handling. Packaging's shall be constructed and closed to prevent any loss of contents that might be caused under normal conditions of carriage by vibration or by changes in temperature, humidity or pressure.

(2) The packaging shall consist of at least three components:

(a) a primary receptacle;

(b) a secondary packaging; and

(c) An outer packaging of which either the secondary or the outer packaging shall be rigid.

(3) Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of carriage, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging shall be secured in outer packaging with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.

(4) For carriage, the mark illustrated below shall be displayed on the external surface of the outer packaging on a background of a contrasting colour and shall be clearly visible and legible. The mark shall be in the form of a square set at an angle of 45° (diamond-shaped) with minimum dimensions of 50 mm by 50 mm; the width of the line shall be at least 2 mm and the letters and numbers shall be at least 6 mm high.

The proper shipping name "BIOLOGICAL SUBSTANCE, CATEGORY B" in letters at least 6 mm high shall be marked on the outer packaging adjacent to the diamond-shaped mark.

(5) At least one surface of the outer packaging shall have a minimum dimension of 100 mm × 100 mm.

(6) The completed package shall be capable of successfully passing the drop test at a height of 1.2 m. Following the appropriate drop sequence, there shall be no leakage from the primary receptacle(s) which shall remain protected by absorbent material, when required, in the secondary packaging.

(7) For liquid substances:

(a) The primary receptacle(s) shall be leak proof;

(b) The secondary packaging shall be leak proof;

(c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;

(d) Absorbent material shall be placed between the primary receptacle(s) and the secondary packaging. The absorbent material shall be in quantity sufficient to absorb the entire contents of the primary receptacle(s) so that any release of the liquid substance will not compromise the integrity of the cushioning material or of the outer packaging;

(e) The primary receptacle or the secondary packaging shall be capable of withstanding, without leakage, an internal pressure of 95 kPa (0.95 bar).

(8) For solid substances:

(a) The primary receptacle(s) shall be siftproof;

(b) The secondary packaging shall be siftproof;

(c) If multiple fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated to prevent contact between them;

(d) If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during carriage then a packaging suitable for liquids, including absorbent materials, shall be used.

(9) Refrigerated or frozen specimens: Ice, dry ice and liquid nitrogen:

(a) When dry ice or liquid nitrogen is used to keep specimens cold, all applicable requirements of ADR shall be met. When used, ice or dry ice shall be placed outside the secondary packaging's or in the outer packaging or an over pack. Interior supports shall be provided to secure the secondary packaging in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or over pack shall be

leak proof. If carbon dioxide, solid (dry ice) is used, the packaging shall be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packaging's and the package (the outer packaging or the over pack) shall be marked "Carbon dioxide, solid" or "Dry ice"'.

(b) The primary receptacle and the secondary packaging shall maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.

(10) When packages are placed in an over pack, the package markings required by this packing instruction shall either be clearly visible or be reproduced on the outside of the over pack.

(11) Infectious substances assigned to UN No. 3373 which are packed and packages which are marked in accordance with this packing instruction are not subject to any other requirement in ADR.

(12) Clear instructions on filling and closing such packages shall be provided by packaging manufacturers and subsequent distributors to the consignor or to the person who prepares the package (e.g. patient) to enable the package to be correctly prepared for carriage.

(13) Other dangerous goods shall not be packed in the same packaging as Class 6.2 infectious substances unless they are necessary for maintaining the viability, stabilizing or preventing degradation or neutralizing the hazards of the infectious substances. A quantity of 30 ml or less of dangerous goods included in Classes 3, 8 or 9 may be packed in each primary receptacle containing infectious substances. When these small quantities of dangerous goods are packed with infectious substances in accordance with this packing instruction no other requirements of ADR need be met.

(14) If any substance has leaked and has been spilled in a vehicle or container, it may not be reused until after it has been thoroughly cleaned and, if necessary, disinfected or decontaminated. Any other goods and articles carried in the same vehicle or container shall be examined for possible contamination.

11 IMRL Transport Labels

(Appendix 2)

DO NOT OPEN THIS BOX ONLY TO BE OPENED IN THE TB LAB IN ST. JAMES'S HOSPITAL	**DO NOT OPEN THIS BOX** ONLY TO BE OPENED IN THE TB LAB IN ST. JAMES'S HOSPITAL	**DO NOT OPEN THIS BOX** ONLY TO BE OPENED IN THE TB LAB IN ST. JAMES'S HOSPITAL
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For the Attention of the I.M.R.L. St. James' Hospital. **ONLY TO BE OPENED IN A CL3 FACILITY**	**For the Attention of the I.M.R.L. St. James' Hospital.** **ONLY TO BE OPENED IN A CL3 FACILITY**	**For the Attention of the I.M.R.L. St. James' Hospital.** **ONLY TO BE OPENED IN A CL3 FACILITY**
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Irish Mycobacteria Reference Laboratory Central Pathology Laboratory St. James's Hospital Dublin 8	**Irish Mycobacteria Reference Laboratory Central Pathology Laboratory St. James's Hospital Dublin 8**	**Irish Mycobacteria Reference Laboratory Central Pathology Laboratory St. James's Hospital Dublin 8**
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12 IMRL WGS pipeline details (Appendix 3)

Once sequencing has been performed (using an Illumina platform), raw fastq files are produced. Various open-source command-line programs are used as part of the IMRL WGS analysis pipeline:

fastp – trims the fastq files according to default quality thresholds

Kraken2 – for detecting contamination within the reads (NTM, nasopharyngeal, human reads)

TBprofiler – identification, lineage calling, resistance prediction according to the WHO mutation catalogue

MTBseq TBfull - identification, lineage calling, resistance prediction according to the WHO mutation catalogue

Ridom Seqsphere – commercial software used for first-line relatedness analysis (for outbreak surveillance)

MTBseq TBjoin – for second-line relatedness analysis (for outbreak surveillance, where a cluster has been detected using Seqsphere)

RaxML – phylogenetic analysis – to outrule contamination on every WGS run

iTOL – for visualisation of phylogenetic trees

snp-dists – for creating NxN matrices of the confirmed clusters of related isolates (to assist in outbreak investigation)

Note: as with all software, version upgrades and updates (minor and major) are added intermittently. Versions are recorded in the IMRL database and major updates are validated using a specific panel of isolates chosen for that purpose.

13 References

1. ADR Regulations January 2023. European Agreement Concerning the International Carriage of Dangerous Goods by Road. https://unece.org/transport/standards/transport/dangerous-goods/adr-2023-agreement-concerning-international-carriage
2. Guidance on regulations for the transport of infectious substances 2021-2022. Geneva: World Health Organization; 2021.
3. Guidelines for the Preparation for Transport of Patient Specimens and other Biological Materials 2019 (www.hse.ie/safetyandwellbeing)
4. Treatment of Nontuberculous Mycobacterial Pulmonary Disease: An Official ATS/ERS/ESCMID/IDSA Clinical Practice Guideline. Clinical Infectious Diseases. 2020
5. CLSI. Susceptibility testing of Mycobacteria, Nocardiae, and other Aerobic Actinomycetes; approved standard. CLSI document M24-A.
6. WHO Consolidated Guidelines on tuberculosis. Module 4. Treatment- drug-resistant tuberculosis treatment. Geneva: World Health Organization;2020. Licence: CC BY-NC-SA 3.0 IGO.

7. WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.