

ST. JAMES'S HOSPITAL LABMED DIRECTORATE		
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Author:: Margaret Fitzgibbon	Date: 20.02.17	
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# Irish Mycobacteria Reference Laboratory

## St James's Hospital

### User Manual

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## The Irish Mycobacteria Reference Laboratory

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 300 mycobacterial cultures per year. The Irish Mycobacteria Reference Laboratory is located in the LabMed Directorate (Central Pathology Laboratory) of St. James's Hospital and is fully accredited to ISO 15189 by the Irish National Accreditation Board (Registration Number 327MT).

### IMRL Services

Services offered by the laboratory include:

- Processing of routine specimens from patients for mycobacteria investigation
- Use of molecular tests on smear positive samples to rapidly identify the presence of mycobacteria and screen for drug resistance in *M.tuberculosis* complex
- Rapid molecular detection of *M. tuberculosis* complex and rifampicin resistance determination performed on smear negative samples upon request by Consultant Microbiologist
- Species identification of mycobacteria using molecular techniques
- Susceptibility testing of *M. tuberculosis* complex to standard anti- mycobacterial drugs
- Rapid detection of MTC and resistance to both Rifampicin and Isoniazid from cultured isolates
- Assistance with isolation of mycobacteria in difficult cases
- Molecular typing and comparison of *M. tuberculosis* complex strains
- Advice to clinicians and laboratories in relation to the diagnosis, treatment and infection control of tuberculosis.
- Referral of resistant *M. tuberculosis* complex isolates for 2<sup>nd</sup> and 3<sup>rd</sup> line drug susceptibility testing
- Referral of NTM isolates for drug susceptibility testing

## Request Forms

### Specimen Request Forms

Specimens sent to the IMRL must originate from the Microbiology/Pathology departments in the client hospital. Hospitals may use their own forms to accompany the samples. Laboratory numbers should be assigned to the forms and samples before they are forwarded to the IMRL. This number will be quoted on all future correspondence from the IMRL.

A request form, in legible writing, must accompany samples and it should contain this minimum set of information.

- Hospital name
- Full name
- Patient's address (non essential, but helpful as patients do switch hospitals)
- Medical Record Number (MRN)
- Date of Birth (DOB)
- Sex
- Site of infection
- Clinical details (Essential if Non-tuberculous Mycobacteria, NTM, are suspected)

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.

### Culture Request Form

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
- Patients address (non essential but helpful as patients are transferred between hospitals)
- Medical Record Number (MRN)

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- Date of Birth
- Sex

The IMRL Culture Request Form can be downloaded from the IMRL website at

(<http://www.stjames.ie/Departments/DepartmentsA-Z/I/IMRL/DepartmentOverview/>)

- IMRL Culture Request Form

## Labelling of Specimens/ Cultures

### 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.

### 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

## Specimens for Mycobacterial Investigations

### Nucleic Acid Amplification Tests

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. Of particular importance at this stage is a test for

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Rifampicin resistance as a marker for MDR-TB. The GeneXpert and HAIN systems that are in use currently both have the potential capability to detect Rifampicin resistance (HAIN also detects Isoniazid resistance) and should be considered in certain circumstances.

The following requirements **must be met** when a NAAT test is requested from an external source (eg. laboratory and/or clinical staff):

### **Specimen Requirements:**

**Sputum/ BAL:** minimum volume of 1 ml

**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

### **Specimens for Culture Investigation**

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. 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 samples 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.

### ***Acceptable Specimens***

The following specimens are acceptable for culture.

- Sputum
- Specimens obtained at Bronchoscopy

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

### *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 sample 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 sample, taken into a sterile plastic container, should be procured and immediately submitted to the IMRL on each of three successive days.

### *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:



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- 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.

### ***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°C if a delay is unavoidable.

### ***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.

### ***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.

### ***Blood***

Blood for mycobacterial culture should be inoculated directly into a BACTEC MycoF/Lytic blood culture bottle according to the manufacturers 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. During internal studies with less than 3 mL of blood, M. intracellulare, M. malmoense, M. haemophilum and M. xenopi exhibited detection delays and-or compromised recovery with BD BACTEC Myco/F Lytic. False positivity most likely will increase when the blood volume is above 5 mL.*

Specimens should be sent to the IMRL 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 get the bottles from the IMRL following an appropriate request. A small supply can be forwarded.

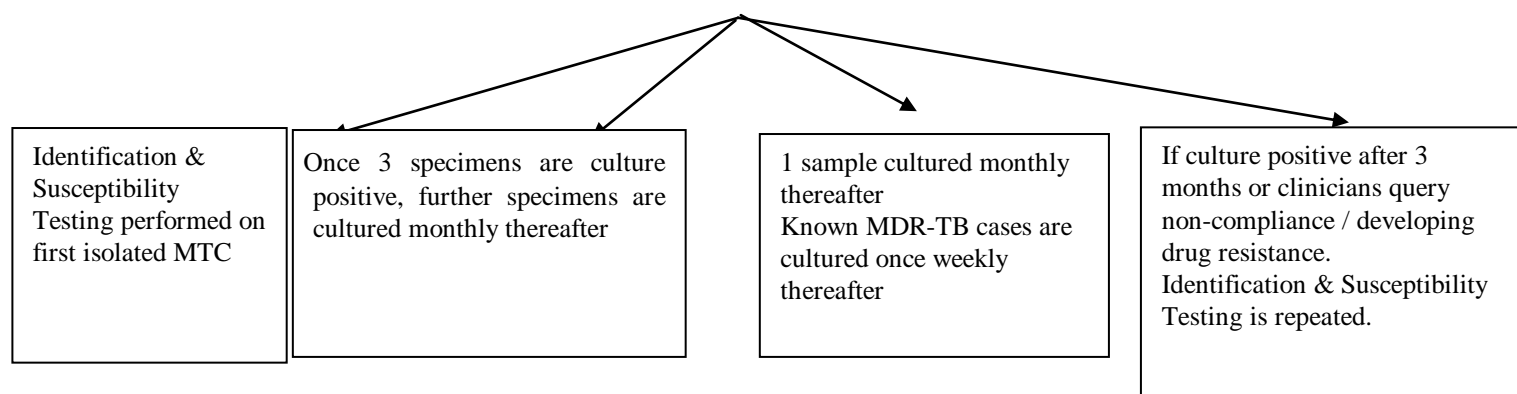
### ***Bone Marrow***

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 IMRL immediately. If microscopy is required the smears should be prepared when the sample is obtained and sent along with the culture material.

## **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 Samples of *Mycobacterium tuberculosis* complex (MTC)**



### **Smear Negative Culture Positive Samples of MTC**

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Identification & Susceptibility Testing performed on first isolated MTC

3 specimens are culture positive, further specimens are cultured monthly thereafter

If culture positive after 3 months or clinicians query non-compliance / developing drug resistance. Identification & Susceptibility Testing is repeated.

### **Note:**

Only **one** ID/SENS is performed **per patient**. Isolates from differing sites are typed. Following typing, if a different strain is isolated 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.

## **Culture Requirements**

In order to minimise turnaround times we are requesting that a minimum of 1-2 mls of positive liquid culture or an LJ slope be sent for examination. **Only pure cultures of mycobacteria will be processed at the IMRL. Contaminated cultures will not be processed.**

Liquid culture should be transferred into a sterile plastic container. 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 containing Middlebrook 7H9 media). In the CAMLIC systems there tends to be large amounts of bacilli present when there are flagged positive. These tend to clump at the bottom of the tube or vial. We have found that if this “sediment” can be aspirated and transferred into a sterile plastic container that we can do rapid molecular testing on the aliquot sent to us directly and we seldom have to wait until a subculture has grown to do initial identification tests. With the exception of LJ slopes glass containers should not be used. The lid should be sealed with parafilm.

## **Packing and Transporting of Infectious Substances**

### **Packing and Transporting of Specimens**

New regulations have come into force, which require specimens to be packaged under U.N. guidelines and transported using a courier service. Specimens of any kind can no longer 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, bijoux 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

### **Packing and Transporting of Cultures**

Changes in the ADR regulations 2009 allow for the transport of MTC by road, under the category UN3373, "Biological Substance, Category B"<sup>1</sup>. Packing instructions P650 apply.

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- 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:

5.

**Irish Mycobacteria Reference  
Laboratory  
Central Pathology Laboratory  
St. James's Hospital  
Dublin 8  
DX6000306 James Street 61E**

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**

## Culture Dispatch

Once the sample has been packaged correctly, send it 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](mailto:imrl@stjames.ie).**

The IMRL fax and email forms can be downloaded from the IMRL website at

(<http://www.stjames.ie/Departments/DepartmentsA-Z/I/IMRL/DepartmentOverview/>)

- Fax form for Culture dispatch

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## Tests Available and Turnaround Times

### Nucleic Acid Amplification Tests

#### *Detection of MTC and Rifampicin Resistance Genes*

Rapid detection of MTC and the detection of Rifampicin resistance associated mutations can be performed directly on clinical specimens. This assay (Cepheid GeneXpert) can be performed daily Monday-Friday.

Please contact the IMRL before dispatching the specimen for NAAT testing.

Requests for NAAT testing on smear negative specimens must be made through a Consultant Microbiologist in the Microbiology Department.

#### *Detection of MTC and Resistance Genes to Rifampicin, Isoniazid, Ethambutol, Fluoroquinolones and Aminoglycosides/ Cyclic Peptides*

Rapid detection of isoniazid, rifampicin, ethambutol, fluoroquinolone (moxifloxacin, ofloxacin) and aminoglycoside (kanamycin, amikacin, capreomycin) resistance genes can be performed on both **smear positive and smear negative pulmonary specimens** and **cultures** referred to the IMRL. This molecular assay is performed weekly on a limited basis. Please contact the IMRL before dispatching isolate/specimen.

### Specimen Service

It is important to note that processing of specimens begins at 8.00am each day. If a sample arrives after this time it will not be processed until 8.00am the following day. It is therefore worthwhile that client laboratories send their specimens to the IMRL as soon as possible.

Samples 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.

### ***Microscopy***

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

### ***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. Current guidelines recommend that a TB laboratory should try to identify *M. tuberculosis* complex within 21 days of specimen receipt and determine susceptibility within 30 days of specimen receipt (Parrish et al, 2011).

### **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.

### ***Identification***

Rapid molecular identification procedures can be applied directly to the culture submitted depending on the quality and quantity of the culture. Identification to MTC level is carried out daily and identification of NTM is performed weekly. The presence of contaminating organisms, (e.g. GNB, GPCs) greatly affects the turnaround times of molecular techniques.

### ***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-tubercle 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 (1.0 µg/ml); Ethambutol (5.0 µg/ml); Pyrazinamide (100 µg/ml).

Susceptibility testing to the following second line anti TB agents is available on request:

Fluoroquinolones: Moxifloxacin (0.25 µg/ml, 2.0 µg/ml); Ofloxacin (2.0 µg/ml)

Aminoglycosides: Kanamycin (2.5 µg/ml), Amikacin (1.0 µg/ml), Capreomycin (2.5 µg/ml)



***Detection of Drug Resistance Mutations to Rifampicin, Isoniazid, Ethambutol, Fluoroquinolones and Aminoglycosides/ Cyclic Peptides***

Rapid detection of isoniazid, rifampicin, ethambutol, fluoroquinolone (moxifloxacin, ofloxacin) and aminoglycoside (kanamycin, amikacin, capreomycin) resistance genes can be performed on cultures referred to the IMRL. This molecular assay is performed weekly on a limited basis. Please contact the IMRL before dispatching the isolate.

***Referral of Isolates for Susceptibility Testing***

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 or the SMRL if further information is required.

Susceptibility testing of resistant MTC isolates for selected 2<sup>nd</sup> and 3<sup>rd</sup> line susceptibility testing is performed in the SMRL and the PHE National Mycobacterium Reference Laboratory, London. There is a minimum turnaround time of two weeks. Please contact the IMRL if further information is required.

***Typing of MTC Isolates***

Molecular typing of all MTC isolates is performed in the IMRL. The turnaround time for this service is 30 days. However, as typing is performed on a weekly basis, urgent analysis can be performed e.g. outbreak situations or possible cases of laboratory cross contamination. In these instances, the turnaround time is 7-10 days. Requests for this service can be made to Prof. Tom Rogers, Dr. Margaret Fitzgibbon or Dr Emma Roycroft or to the laboratory directly.

## Telephone Policy

Phoning the IMRL for results should be kept to a minimum. One phone call, around 4.00pm, is usually sufficient to get the microscopy results for each working day, if required. Our Medical staff will phone all **positive** results and record the details in our Telepath LIS system. Client laboratories should provide contact details including name(s) and 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. The TB laboratory can be contacted during the day at (01) 4284211.

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

1. Isolate identification.
  - a. Identification to MTC level. Further speciation will follow.
  - b. Identification of NTM.
  - c. Rapid detection of MTC and isoniazid and rifampicin resistance genes by molecular methods.
2. Susceptibility tests
  - a. For MTC isolates the susceptibility test results follow the initial identification.
  - b. Susceptibility test for NTM are performed by request in the SMRL. The IMRL will issue a final report when received from the SMRL.
  - c. Selected 2<sup>nd</sup> & 3<sup>rd</sup> line susceptibility testing (for resistant MTC isolates where applicable).
3. *M. tuberculosis* complex typing

An interim report is issued with the final identification of the organism and susceptibility tests results until molecular typing is complete. Typing results are considered the final report at the IMRL. Typing results are reported electronically through CIDR to individual Public Health departments. Currently, group typing reports for each institution are issued by the IMRL on a yearly basis.

## Microscopy

The First Smear Positive result for a patient will be phoned immediately by our Medical staff to the contact number supplied by the client laboratory. Our staff will record details of this contact. Negative results will not be phoned.

## Culture

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

## Identification and Susceptibility

The Identification of a patients' First Isolate will be phoned immediately.

Susceptibility test results will be phoned immediately.

Our medical staff will record details of each contact.

## 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 typing is complete. Typing results are considered the final report at the IMRL and are reported electronically.

## 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”**

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## 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

## Protocol for Urgent TB Microscopy “Out of Hours”

Please Note: Urgent microscopy “Out of hours” 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

Central Pathology Laboratory

St James’s Hospital

## Time Limits for Requesting Additional Examinations

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

## Contact Details

Name and Designation	Telephone Numbers & E-mail Address
Prof. Tom Rogers Clinical Director	(01) 8962131, 4284611 <a href="mailto:trogers@stjames.ie">trogers@stjames.ie</a> / rogerstr@tcd.ie
Dr Margaret Fitzgibbon Chief Medical Scientist	(01) 4162963 mfitzgibbon@stjames.ie
Dr Emma Roycroft Specialist Medical Scientist	(01) 4284211 eroycroft@stjames.ie
Ms Lorraine Montgomery Senior Medical Scientist	(01) 4284211 lmontgomery@stjames.ie
IMRL direct phone line	(01) 4284211, 4162980 imrl@stjames.ie

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

Prof. Tom Rogers

Prof. Joseph Keane (01 410 3920)

**Advice on scientific matters can be obtained from**

Dr Margaret Fitzgibbon

Dr Emma Roycroft

Ms Lorraine Montgomery

**Postal Address:**

Irish Mycobacteria Reference Laboratory,

Microbiology Department,

LabMed Directorate,

St. James's Hospital,

Dublin 8

## Appendix 1

### Packaging Instruction P650

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.

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- (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

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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.



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	<b>Appendix 2</b>  <b>IMRL Labels for Transport</b>	
<b><u>DO NOT OPEN THIS BOX</u></b>  <b>ONLY TO BE OPENED IN THE <u>TB LAB</u> IN ST. JAMES'S HOSPITAL</b>	<b><u>DO NOT OPEN THIS BOX</u></b>  <b>ONLY TO BE OPENED IN THE <u>TB LAB</u> IN ST. JAMES'S HOSPITAL</b>	<b><u>DO NOT OPEN THIS BOX</u></b>  <b>ONLY TO BE OPENED IN THE <u>TB LAB</u> IN ST. JAMES'S HOSPITAL</b>
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<u><b>For the Attention of the I.M.R.L. St. James' Hospital.</b></u>	<u><b>For the Attention of the I.M.R.L. St. James' Hospital.</b></u>	<u><b>For the Attention of the I.M.R.L. St. James' Hospital.</b></u>
<b>ONLY TO BE OPENED IN A <u>CL3</u> FACILITY</b>	<b>ONLY TO BE OPENED IN A <u>CL3</u> FACILITY</b>	<b>ONLY TO BE OPENED IN A <u>CL3</u> FACILITY</b>
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<b>Irish Mycobacteria Reference Laboratory</b> <b>Central Pathology Laboratory</b> <b>St. James's Hospital</b> <b>Dublin 8</b> <b>DX6000306 James Street 61E</b>	<b>Irish Mycobacteria Reference Laboratory</b> <b>Central Pathology Laboratory</b> <b>St. James's Hospital</b> <b>Dublin 8</b> <b>DX6000306 James Street 61E</b>	<b>Irish Mycobacteria Reference Laboratory</b> <b>Central Pathology Laboratory</b> <b>St. James's Hospital</b> <b>Dublin 8</b> <b>DX6000306 James Street 61E</b>
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