ST. JAMES'S HOSPITAL LABMED DIRECTORATE			
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Irish Mycobacteria Reference Laboratory

St James's Hospital

Specimen Service

Specimen Request Form

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
- Medical Record Number (MRN)
- Date of Birth (DOB)
- Sex
- Site of infection
- Clinical details (Essential if Non-tuberculous Mycobacteria, NTM, are suspected.)

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.

And (non essential, but helpful as patients do switch hospitals)

- Patient's address

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.

Specimens for Culture

Guidelines given here are for those institutions that the IMRL has agreed to provide a culture service for. The IMRL does not provide a **routine** Nucleic Acid Amplification service for outside agencies, please refer to Appendix 1 for use of this service.

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)

Unacceptable Specimens

- Poor quality sputum specimen's e.g. salivary specimens or specimens of minute quantities.
- Faeces.
- 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:

- 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^{o}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 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. Add 5-10ml of blood to the bottles using an aseptic technique. 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 0.5 ml should be taken into a plastic sterile universal container. Specimens of greater than 0.5 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.

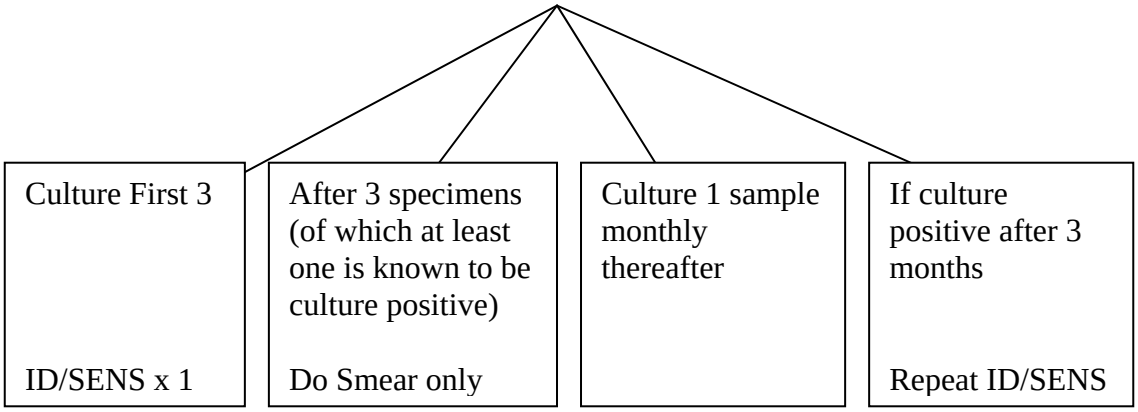
In General:

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 transport in the same bag. Transportation of samples to the laboratory must occur in a safe manner and comply with the appropriate regulations. Appendix 2

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.

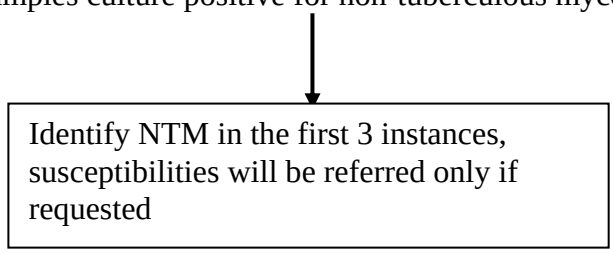
Smear Positive Samples of *Mycobacterium tuberculosis* complex (MTC) from Same Site



Culture positive MTC from each different site will get full ID/SENS.

Smear Negative Culture Positive Samples of MTC from Same Site

Samples culture positive for non-tuberculous mycobacteria (NTM)



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 2

Packaging of Specimens

- 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

Reporting

Telephone Policy

Phoning the IMRL for results should be kept to a minimum. One phone call, around 4.30pm, 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. 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 following policies will be adopted for reporting positive results by telephone.

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.

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"**

Contact Details

Clinical Director	**Chief Medical Scientist**	**Specialist Grade Medical Scientist**
Prof. Tom Rogers	Mr. Noel Gibbons	Dr Margaret Fitzgibbon
Tel: (01) 6082138	(01) 4162963	(01) 4162980

Senior Medical Scientist

Ms Lorraine Montgomery

(01) 4162980

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 medical scientist as outlined below, who will then provide the phone number of a Consultant. The decision will then be communicated back to the medical scientist by the consultant.

"On Call" Contact Details

During Sessions:	Mon –Fri:	5.00p.m. -10.00p.m.
	Sat/Sun	9.00a.m. –10.00p.m.

Phone 4162972 or 4162046. If no answer please call the SJH switchboard at 4103000

On Call after 10.00p.m.
Please call the SJH switchboard on 4103000 and ask for the medical scientist "On Call" in microbiology on bleep number 194
At this stage discuss the urgent requirement with the medical scientist.

If approved:
Send the specimen, appropriately packaged, to:
Microbiology Department
Central Pathology Laboratory
St James's Hospital

Turnaround Times

It is important to note that processing of specimens begins at 9.00am each day. If a sample arrives after this time it will not be processed until 9.00am 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 despatched to the IMRL by 3.00pm Friday or Saturday morning, microscopy results will be available by 4.30pm Monday. If these specimens are held in the client laboratory until Monday morning the microscopy result will not be available until Tuesday at 4.30pm.

Microscopy

Microscopy results are available within 24 hours of receipt of the specimen in the IMRL on any working day.

Specimens received after 9.00am Friday and before 9.00am Monday will have microscopy results available on Monday after 4.30pm.

Culture

All specimens are incubated for 6-8 weeks. Microscopy positive samples that are culture negative after 6 weeks incubation are incubated for a further 2 weeks. If AN isolate has NOT been recovered from this patient at any stage before, the specimen will be sent for Nucleic Acid Amplification studies in an effort to determine if the bacilli are members of the *M.tuberculosis* complex.

The following tables indicate the time it has taken to detect cultures that have been culture positive for MTC. This varies most considerably depending on whether the culture is positive or negative by microscopy.

The CDC guidelines recommend that a TB laboratory should try to identify *M.tuberculosis* within 10-14 days of specimen receipt and determine susceptibility within 15-30 days of specimen receipt. Since 2005 various rapid molecular techniques for identification of *Mycobacteria* sp. have been introduced on a phased basis in the IMRL. These tests are used to identify all *Mycobacteria sp*. and are currently performed daily and weekly. Together, all the tests give us an improved capacity to identify MTC isolates more frequently thus further reducing the turnaround times.

Microscopy Results

In specimens with disease involving a pulmonary component, 70%, 57% and 83% were smear positive in 2008, 2009 and 2010 respectively. The smear positive rate for **patients** with disease involving a pulmonary component was 60%, 50% and 70% in 2008, 2009 and 2010 respectively.

Turn-around Times for Culture

Times are from day of specimen receipt to culture becoming positive

Smear positive Culture positive *M.tuberculosis* complex (MTC)

Auramine Positive MTC	2005	2006	2007	2008	2009	2010
Median Days (n)	9(41)	8 (62)	7 (57)	7 (71)	7 (50)	10 (46)
%age Positive @21 days	83%	85%	91%	87%	88%	98%

(n) is the number of patients

Smear Negative Culture positive *M.tuberculosis* complex

Auramine Negative MTC	2005	2006	2007	2008	2009	2010
Median Days (n)	21(14)	21(24)	16(36)	15(38)	21(39)	19 (44)
%age Positive @28 days	64%	74%	89%	87%	64%	72%

(n) is the number of patients

Time to Culture Positivity for all ***M.tuberculosis*** complex patients

Time to Culture Positive for MTC Patients	2005	2006	2007	2008	2009	2010
Median Days (n)	14 (55)	14 (86)	14 (92)	14 (109)	14 (91)	14 (91)
%age Positive @ 21 days	76%	77%	86%	79%	73%	84%

(n) is the number of patients

Turnaround Times for Identification and Susceptibility of MTC

Times are from day of specimen receipt to report for each **Patient**

Time to Identification of MTC	2005	2006	2007	2008	2009	2010
Median Days (n)	28 (74)	21 (102)	18 (112)	20 (139)	21 (94)	20 (88)
%age Identified @ 28 days	53%	69%	82%	78%	74%	76%

(n) is the number of patients

Time to Susceptibility for MTC isolates	2005	2006	2007	2008	2009	2010
Median Days (n)	37 (66)	26 (96)	28 (99)	29 (115)	30 (96)	29 (84)
%age Performed @ 42 days	56%	77%	84%	89%	74%	79%

Turnaround Times for Typing of MTC

Typing has been performed on all isolates recovered and received for the year 2010. This is an ongoing process and we cannot state exact turnaround times at this moment. However, as typing is performed on a weekly basis urgent analysis can be performed, e.g. in outbreak situations or in possible cases of laboratory cross contamination. In these instances the turnaround time is 7-10 days. Requests for this service can be made to Noel Gibbons or Dr Margaret Fitzgibbon at the above contact numbers. If you wish to get a report of those isolates that have been typed for your institution please contact Dr Margaret Fitzgibbon.

Appendix 1

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

Laboratories requiring NAAT should contact the Consultant microbiologist or the Chief Medical Scientist to discuss any requests which will be provided on a strictly limited basis. There is also an additional charge for this service as the laboratory is currently not funded for this service.

Appendix No. 2: 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 packagings in such a way that, under normal conditions of carriage, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packagings shall be secured in outer packagings 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 in 6.3.2.5 as specified in 6.3.2.2 to 6.3.2.4 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 packagings 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.