

Irish Mycobacteria Reference Laboratory

St James's Hospital

Culture Service

2008

# 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
- Medical Record Number (MRN)
- Date of Birth
- Sex

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

- Patients address

## Packing and Transporting of Cultures

Changes in the ADR regulations 2007 allow for the transport of MTC by road, under the category UN3373, “Biological Substance , Category B” . Packing instructions P650 apply.

Briefly:

### Packaging of Cultures

- In order to minimise turnaround times we are requesting that a minimum of 2.5 ml of positive liquid culture or an LJ slope be sent for examination. Liquid culture should be transferred into a sterile plastic container. 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.
- 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:

**Only to be opened in a CL3 facility**

Irish Mycobacteria Reference Laboratory

Microbiology Department

C.P.L.

St James's Hospital

Dublin 8

## Culture Dispatch

Once the sample has been packaged correctly:

Send it to the IMRL by courier.

Please **Fax** the IMRL (01) 4284351 to state that a culture is on the way, (sample form included, Appendix 2).

## Turnaround Times

The turnaround time for the identification and susceptibility will be greatly affected by the concentration of organisms in the culture received.

### Identification

Presently, rapid molecular identification procedures are applied directly to the culture submitted. These do not always contain an amplification step e.g. Accuprobe MTC, and therefore the result can depend on the quality and quantity of the culture submitted. It is the goal of the IMRL to perform molecular techniques twice a week. This should generally result in a maximum time of 7 days for non-tuberculous mycobacteria (NTM) but less than 4 days for Mycobacterium complex (MTC).

The table below shows the turnaround times to complete identification and susceptibility tests for cultures of *M.tuberculosis* referred to the IMRL over the past few years.

Time to Identification	2004	2005	2006
Average Days	8	4	7
Minimum Days	1	1	1
Maximum Days	29	12	16
Median Days	6	3	7

Time to Susceptibility	2004	2005	2006
Average Days	17	20	16
Minimum Days	7	9	10
Maximum Days	43	81	51
Median Days	15	15	14

## Susceptibility

Following receipt of cultures a new inoculum is prepared and when sufficient growth is obtained, (usually 4-5 days), the susceptibility tests to the first line anti-tubercle drugs Isoniazid, Rifampicin, Ethambutol and Pyrazinamide are performed (Streptomycin is also tested but this is not a first line drug). These tests also take 5-7 days to complete. Ideally the process takes <14 days.

## Telephone Policy

Phoning the IMRL for results should be kept to a minimum. The Medical staff in the Microbiology department will phone all **positive** results. 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 4162980.

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

1. Isolate identification.
  - a. For members of the *M.tuberculosis* complex (MTC) the first report will state that the isolate has been identified as an MTC. Members of the MTC include *M.tuberculosis*, *M. bovis*, *M bovis* BCG, *M.africanum*, *M. caprae*, *M. microti* and *M. canettii*. Further speciation will follow but this can take up to 3 weeks in some cases.
  - b. Nontuberculous mycobacteria can usually be identified by our current molecular technique with full and final identification. Very occasionally the isolate may not identify by this technique and will need to be referred abroad for identification. This test is performed on at least a weekly basis.
2. Susceptibility tests
  - a. For MTC isolates the susceptibility test results follow the initial identification. In the majority of cases the final identification of *M.tuberculosis* is also made at this time and a final report is issued.
  - b. Susceptibility test for NTM are performed according to the criteria outlined in Appendix 2 or by request.
3. Final Identification

## Contact Details

<b>Clinical Director</b>	<b>Chief Medical Scientist</b>	<b>Senior Medical Scientist</b>
Prof. Tom Rogers	Mr. Noel Gibbons	Ms Lorraine Montgomery
Tel: (01) 6082138	(01) 4162969	(01) 4162980

# Appendix 1

## Fax Form for Culture Dispatch to IMRL

Fax Number for IMRL (Microbiology St. James' Hospital) 01 4284351

The following **culture** has been dispatched to the IMRL

Patient Initials.....MRN.....D.O.B.....

Laboratory Number.....

Date dispatched.....

Time dispatched.....

Courier.....

Sent by: (block Letters).....

Signature:.....

# Irish Mycobacteria Reference Laboratory

## Request Form

### Test Requested

**Culture: Please perform Identification and Susceptibility Tests**

Patients Surname.....Forename.....

Hospital Number.....Date of Birth.....M/F

Address.....

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Nationality.....Country of Origin.....

Name of Hospital Sending Culture.....

External Laboratory Number.....

Microbiology/Pathology Consultant.....

Contact Phone number.....

Specimen Type.....

Culture Media/System Used (e.g. MGIT 960).....

Clinical Details.....

HIV status.....Pos/Neg

MDR-TB Contact.....Yes/No

**Comments.....**

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## Appendix 2

### IMRL Susceptibility Testing algorithms for Non-Tuberculous Mycobacteria (NTM)

#### Introduction:

Before pursuing susceptibility tests the laboratory the relevance of the isolate should be established using a combination of parameters from both the British Thoracic Society<sup>1</sup> (BTS) and American Thoracic Society<sup>2</sup> (ATS). Good communication between the clinician and the laboratory is essential, combined with adequate specimens and appropriate clinical details.

The British Thoracic Society suggests that the significance of an isolate can only be established by considering:

- The type of specimen from which the Mycobacterium was isolated.
- The number of isolates.
- The degree of growth.
- The identity of the organism.

In general multiple isolates are needed from non-sterile sites to establish disease whereas one positive culture from a sterile site, particularly where there is supportive histopathology, is usually significant. The clinical presentation and any predisposing factors are also helpful.

The American Thoracic Society currently recommends the following bacteriologic criteria for determining the clinical significance of NTM from respiratory specimens:

- A.** If 3 positive sputa/bronchial wash results are available from the previous 12 months:
- 3 positive cultures with negative smears results for Acid Fast Bacilli  
**or**
  - 2 positive cultures and one positive AFB smear
- or*
- B.** If only one bronchial wash is available:
- A positive culture with a 2+, 3+, 4+ AFB smear or 2+, 3+, 4+ growth on solid media.
- or*
- C.** If sputum /bronchial wash evaluations are non-diagnostic or another disease cannot be excluded:
- Transbronchial or lung biopsy yielding a NTM  
**or**
  - Biopsy showing mycobacterial histopathologic features (granulomatous inflammation and/or AFB) and one or more sputa or bronchial washing are positive for an NTM even in low numbers.

The following first line susceptibilities to various NTMS are derived from guidance published by ATS<sup>2</sup> and NCCLS<sup>3</sup> and will be performed if clinically required.

## 1. Susceptibility Testing of *M. avium* complex (MAC)

### Indications for performing susceptibility tests (NCCLS, ATS)

MAC isolates should be tested in the following situations:

- Clinically significant isolates from patients on prior macrolide therapy
- Isolates from patients who develop bacteraemia while on macrolide prophylaxis
- Isolates from patients who relapse while on macrolide therapy
- Initial isolates from blood, tissue or from clinically significant respiratory samples

Susceptibility testing should be repeated after 3 months of treatment for patients with disseminated disease and after six months of treatment for patients with chronic pulmonary disease, if the patient shows no clinical improvement or clinical deterioration and is still culture positive

### **Laboratory Susceptibility Testing.**

**Method:** Radiometric Bactec 460TB

**Antimicrobials to be tested:** Clarithromycin only

## 2. Susceptibility Testing of *M. kansasii*

### Indications for performing susceptibility tests

- All clinically significant initial isolates.
- Failure to convert to smear and/or culture negative.
- Relapse.

### Laboratory Susceptibility Testing.

**Method:** Radiometric Bactec 460TB

**Antimicrobials to be tested:** Rifampicin only.

Repeat susceptibility if the patient remains culture positive after 3 months of appropriate therapy.

If rifampicin resistant, then test the following antimicrobials:

- Ethambutol
- Isoniazid
- Streptomycin
- Clarithromycin
- Amikacin
- Ciprofloxacin

There is limited experience with the concentration of these drugs and any of the test media for isolates of *M. kansasii*.

### 3. Susceptibility testing of Rapidly Growing Mycobacteria

The rapidly growing mycobacteria include the following:

- *M. fortuitum* group
- *M. chelonae*
- *M. abscessus*
- *M. goodii*
- *M. mucogenicum*
- **M. smegmatis**

Indications for performing susceptibility tests:

- Clinically significant isolate e.g., isolates from blood, tissue and skin and soft tissue lesions.
- Respiratory isolates if recovered from multiple specimens and in large numbers i.e. smear positive.
- Clinical failure to eradicate rapidly growing mycobacteria from almost any site (except respiratory) after six months of appropriate antimicrobial therapy

#### Laboratory Susceptibility Testing

**Method**<sup>4,5,6</sup>: Etest™ (strip diffusion MIC)

**Antimicrobials to be tested:**

- Amikacin
- Ciprofloxacin
- Clarithromycin
- Imipenem
- Rifampicin
- Ethambutol

## 4. Susceptibility testing of *M. marinum*

Routine susceptibility testing of this species is not recommended.

Susceptibility tests may be performed if patients fail several months of therapy and remain culture positive.

### **Laboratory Susceptibility Testing**

**Method:** Radiometric 460TB

#### **Antimicrobials to be tested:**

- Rifampicin
- Ethambutol
- Clarithromycin
- Amikacin

## 5. Miscellaneous Slowly Growing Mycobacteria

### a) Non-fastidious Species

These include *M. xenopi*, *M. malmoense*, *M. simiae*, *M. terrae*.

#### Laboratory Susceptibility Testing

**Method:** There is no recommended standard methodology. The radiometric Bactec 460TB will be used.

**Antimicrobials to be tested:** Testing should include the same primary and secondary drugs used for *M. kansasii*.

### b) Fastidious Species

These include *M. haemophilum*, *M. genavense*, *M. ulcerans*

#### Laboratory Susceptibility Testing

**Method:** There is no recommended standard methodology. If susceptibility is required, the radiometric Bactec 460TB will be used.

#### Antimicrobials to be tested:

- Rifampicin
- Ethambutol
- Clarithromycin
- Ciprofloxacin

Note: There may be specific individual clinical circumstances where additional susceptibility tests would be beneficial and the laboratory will endeavour to facilitate but these situations will need to be discussed with the laboratory director.

## References

1. Management of opportunist mycobacterial infections: Joint Tuberculosis Committee guidelines 1999. Subcommittee of the Joint Tuberculosis Committee of the British Thoracic Society *Thorax* 2000;**55**: 210–218
2. American Thoracic Society diagnosis and treatment of disease caused by nontuberculous mycobacteria. *American Journal of Respiratory and Critical Care Medicine* 1997; **156**: Suppl.2 S1-S25.
3. NCCLS. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard. NCCLS document M24-A. NCCLS, 940 West Valley Road, Wayne, Pennsylvania.
4. Bihle, J.R. et al. Evaluation of E-test for Susceptibility Testing of Rapidly Growing Mycobacteria. *J Clin Microbiol* 1995; **33**: 1760-1764.
5. Hoffner, S.E. et al. Evaluation of E-test for Rapid Susceptibility Testing of *M. chelonae* and *M. fortuitum*. *J Clin Microbiol.* 1994; **32**: 1846-1849.
6. Koontz F.P. et al. E-test for Routine Clinical Antimicrobial Susceptibility Testing of Rapid Grower Mycobacterial isolates. *Diag Microbiol Infect Dis.* 1994;**19**: 183-186