

Standard Operating Procedure on *Mycobacterium sp.* Culture and PCR

Sampling for patients with suspected Tuberculosis/NTM infection

- Tuberculosis is a disease caused by members of the *Mycobacterium tuberculosis* complex (MTBC) whilst environmental non-tuberculous mycobacteria (NTM) cause a range of diseases in susceptible individuals.
- Pulmonary disease predominates but disseminated disease may occur at any deep tissue site.
- *M. chimera* infection has been linked to previous cardiac surgery and time on cardiopulmonary bypass machines and should be considered in anyone with granulomatous pathology and a history of cardiac surgery.
- AFB smear and culture should be undertaken on biopsy samples from patients with lesions suggestive of TB/NTM infection radiologically and/or risk factors for disease.
- Gene-Xpert is a PCR that detects members of the MTBC and a marker of Rifampicin resistance.

Specimen Collection

- Where possible this should be performed prior to the initiation of anti-tubercular treatment
- Aerosol generating procedures such as sputum induction and bronchoscopy must be performed in an appropriately engineered and adequately ventilated area (See ICT policy)
- Sterile samples kept for >1 hour prior to transport should be refrigerated

Suitable containers:



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Sample Type	Collection requirements
Sputum	<ul style="list-style-type: none"> • 3 samples >5ml 24 hours apart (see ICE request 1 to avoid rejection of samples with the same date label or ask patient to date labels) • Purulent samples have a higher yield, consider induction with saline nebs in patients with a dry cough
Bronchoalveolar lavage/bronchial washings	<ul style="list-style-type: none"> • Avoid contamination of bronchoscope with tap water as may contain environmental <i>Mycobacteria sp</i> • Samples from a protected catheter tip are best avoided
Gastric washings	<ul style="list-style-type: none"> • 3 samples >5ml 24 hours apart • Performed in young children who swallow their secretions • Send fresh to avoid degradation by acid contents
Urine	<ul style="list-style-type: none"> • Do not use boric acid tubes, use sterile universal (see above) • Early morning urines have greater yield but MSU acceptable
Sterile fluids (CSF, pleural, ascitic etc)	<ul style="list-style-type: none"> • Large volume (>6ml) required for culture • If diagnosis suspected after initial cell counts, repeat for a larger volume • Pleural/pericardial/ascitic fluid have low culture yield and concurrent biopsy should be considered
Pus	<ul style="list-style-type: none"> • Do not use swabs • Collect aseptically and place In sterile universal (see above)
Blood	<ul style="list-style-type: none"> • Mycobacterial blood culture bottles available in pathology on request
Bone marrow	<ul style="list-style-type: none"> • Do not place in an EDTA tube – inhibits growth of mycobacteria • Use specific Mycobacterial blood culture bottles from pathology • Discuss with microbiology if other disseminated infections suspected (leishmaniasis, histoplasmosis etc).
Sterile tissue biopsy	<ul style="list-style-type: none"> • Place in a sterile universal (see above) covered in sterile saline • Samples in formalin will not be processed • Large, caseous samples are ideal but smaller samples may be pooled in a single container for homogenisation in the laboratory

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ICE Specimen requests and labelling

Individual sample requests – made on 'Infection Science' page, 'Bacteriology' tab

The screenshot shows the 'Bacteriology' tab in the ICE system. The left sidebar contains navigation links like 'Patient Search', 'Discharge', 'Manuals', 'Reporting', 'Requesting', 'New Request', 'View Requests By Patient', 'View Requests By Location', 'Service Provider List', 'Deferred Orders List', 'Bookings Assistant', 'Sample Collection', 'OpenNet Ward Requests', and 'OpenNet Patient Requests'. The main panel is divided into sections for 'Blood culture (2 bottle set) MCS', 'Tissue sample for MCS', 'Respiratory', 'Enteric, Genital and Urine samples', 'Cross Infection', and 'Most recent requests made for this patient:'. The 'Respiratory' section is circled in red, showing options for 'BAL/PC for MCS', 'Pleural fluid for MCS', 'Sputum/Respiratory samples for MCS', 'TB', 'TB/Mycobacteria', 'TB/Mycobacteria Urine', and 'TB/Mycobacteria for Blood and BM'. The 'Most recent requests made for this patient:' table lists several requests with details on the date, time, investigations, priority, location, ordered by, and status.

Requested	Investigations	Priority	Loc	Ordered	Status
13 Sep 2019 08:12:53	CRP, FBC, U&E and Creatinine, Liver function test	1. Routine	BRI A800	LeandroLor	RR
10 Sep 2019 15:19:58	Vitamin B12, Calcium, CRP, FBC, Serum Folate, Magnesium, Phosphate, U&E and Creatinine, Liver function test, Thyroid function test	1. Routine	BRI A800	LeandroLor	RR
09 Sep 2019 15:56:59	Calcium, CRP, FBC, Magnesium, Phosphate, U&E and Creatinine, Liver function test	1. Routine	BRI A800	LeandroLor	RR
08 Sep 2019 16:23:11	FBC, Magnesium, Phosphate, U&E and Creatinine, Liver function test	1. Routine	BRI A800	GallardoE	RR
07 Sep 2019 13:31:24	CRP, FBC, Magnesium, Phosphate, U&E and Creatinine	1. Routine	BRI A800	BriarD	RR

Set of 3 sputum/urines – made on 'Infection Science' page, 'Profiles' tab (allows 3 samples to be sent without labels having the same date)

The screenshot shows the 'Bacteriology' tab in the ICE system, specifically the 'Profiles' section. The left sidebar is the same as in the previous screenshot. The main panel shows various profile options like 'Antenatal screen', 'Blood Borne Virus Serology', 'BMT pre-PBSC collection', 'BMT PCR virology', 'BMT workup', 'Congenital CMV storage sample', 'CSF PCR virology', 'Egg Donor', 'Fetal Abnormality Investigations', 'Guillaine Barre syndrome serology', 'Hepatitis/Abnormal LFTs', 'Supplementary Hepatitis/Abnormal LFT's', 'Infertility female screen', 'Infertility male screen/Sperm storage', 'Lymphadenopathy/Glandular Fever', 'MMR and chickenpox immunity', 'Organ donor', 'Pneumonia/Respiratory tract infection', 'Pre-immunosuppression screen', 'Rash Contact', 'Rash Diagnosis', 'Renal transplant workup', 'Sexually transmitted infections', and 'Viral Ophthalmology'. The 'Bacteriology profiles' section is circled in red, showing options for 'TB - Sputum Screen Set' and 'TB - Urine Screen Set'. The 'Most recent requests made for this patient:' table is also present at the bottom.

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Authors: Jennifer Tomlins Consultant Microbiologist; Rajeka Lazarus, Consultant Microbiologist

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Gene-Xpert Testing

- Automatically performed on first smear positive sputum
- May be requested on children <15 with smear negative sputum
- May be requested on smear negative sputum, BAL, fluid and tissue samples if the patient has been reviewed by a member of the microbiology/ID/TB team AND satisfies one of the following criteria:
 - The patient is HIV positive
 - Rapid information on mycobacterial species would alter the patient's care
 - The need for a large-scale contact tracing initiative is being explored
 - The patient has risk factors for MDR TB
- There is no ICE/millennium request – add to existing samples by contacting the microbiology SpR/consultant

Results

- Microbiology will report microscopy results within 24 hours of receipt
- Gene-Xpert testing usually takes 24-36 hours following the request (except at weekends)
- Samples will be cultured for a total of 6 weeks, after which a negative report will be automatically generated
- Positive results will be communicated to the requesting physician, ensure the responsible team are named on the request.