



**INTEGRATED PATHOLOGY SERVICE
MICROBIOLOGY DOCUMENT
WEST SUSSEX**

**Pathology User Manual
Microbiology Investigations
Tuberculosis
[PD-MIC-UMTB]**

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Investigations for Tuberculosis (TB)

For the initial diagnosis of mycobacterial infection all specimens should be fresh and taken, whenever possible, before anti-tubercular treatment is started. 'Other' antimicrobials may also have significant anti-mycobacterial activity, notably the fluoroquinolones such as ciprofloxacin, levofloxacin or moxifloxacin, and the macrolides such as clarithromycin or azithromycin.

Sputum

Sputum specimens should be relatively fresh (less than 1 day old) to minimise contamination. Purulent specimens are best. Three samples of $\geq 5\text{mL}$ should be collected approximately 8-24 hours apart with at least one from early morning. Samples taken early morning (i.e. shortly after patient waking) have the greatest yield. When the cough is dry, physiotherapy, before expectoration may be helpful

Bronchoalveolar lavage

These may be sent if spontaneous or induced sputum is unavailable.

Note: Minimum sample size is preferably 5mL.

Tap water may contain environmental *Mycobacterium* species.

Early morning urines

Urine specimens should be collected in the early morning on three consecutive days in a designated CE marked leak proof 250ml silver-topped container (that does not contain boric acid). Information sheets are available from the Trust intranet detailing correct specimen collection protocol and transported to the laboratory in the standard microbiology request form. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable, but not ideal alternative.

Gastric washings

Gastric washings are usually used for children where there are problems obtaining sputum. Young children will often swallow their respiratory secretions rather than cough them up. Induced sputum is considered preferable to gastric washings, if possible. Collect samples early in the morning (before breakfast) on 3 consecutive days. Preferably, a minimum volume of 5mL should be collected. Aspirates should be promptly delivered and processed to avoid acidic deterioration of organisms. Results of direct microscopy on gastric washings can be misleading because other acid- fast bacilli are normally present in the stomach.

Sterile site body fluids

Collect aseptically as much CSF sample as possible into a plain aseptically produced container without preservatives and send to the laboratory in a sealed plastic bag. If only a small volume is available after initial lumbar puncture, and the findings of cell counts and protein suggest TB meningitis, a second procedure should be considered to obtain a larger volume to improve chances of achieving positive cultures.

It should be noted that pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis*, and that a concurrent pleural or pericardial biopsy taken with the fluid is more useful. A negative result on these fluids does not rule out the diagnosis.

Tissue and Bone

Specimens should be collected aseptically and placed in a plain aseptically produced container without preservatives and sent to the laboratory in a sealed plastic bag. A caseous portion should be selected if possible: the majority of organisms will be found in the periphery of a caseous lesion.

Blood Specimens

Any requests for *Mycobacterium avium/intracellulare* culture from blood should be submitted in two lithium heparin blood tubes (referred investigation). Please note a blood sample (either in vacutainer or blood culture bottles) is not appropriate for routine *M. tuberculosis* investigation.

Atypical Mycobacteria

If suspected, please note this on the request form.

Limitations of examination procedure

- Automated liquid culture systems available in the UK have been tested for their ability to detect a wide range of both slow and rapidly growing mycobacteria, however reliance must not be placed on these systems alone for the isolation of all mycobacterial species, particularly when investigating patients who are immunocompromised. Their limitations lie in a single incubation temperature and the difficulty of providing the growth additives necessary for certain very fastidious species. Advice may be sought from the Reference Laboratories or relevant system manufacturer. Rare isolates of *M. tuberculosis* are recovered only on egg-based media, such as a Lowenstein Jensen slope.
- On rare occasions organisms may be encountered that grow in the MB/BacT growth medium but do not produce enough carbon dioxide to be determined as positive. For instance, samples from patients undergoing treatment with anti-mycobacterial agents may show impaired growth.

- Cultures obtained for primary diagnosis after initiation of antimicrobial therapy may produce negative results.
- Recovery of mycobacteria in the MB/BacT bottle is dependent on the quality of the specimen collected, the number of culturable organisms in the specimen volume and the method of processing. Adherence to procedural instructions is critical for optimum recovery of mycobacteria
- Pleural or pericardial fluids are not very sensitive samples for the detection of *M. tuberculosis*. A concurrent pleural or pericardial biopsy taken with the fluid is more useful. A negative result on these fluids does not rule out the diagnosis
- Ideally urine specimens are collected in the early morning on 3 consecutive days in a CE marked leak proof container. If there are no appropriate containers for a whole Early Morning Urine (EMU) sample, a midstream EMU sample is an acceptable alternative.

IGRA (Interferon Gamma Release Assay) T-Spot

Unless funded by the requesting department, requests for T-Spot that fall within NICE guidelines will be considered up on discussion with a Consultant Microbiologist.

Requesters please note: - Requests for T-Spot require a blood sample taken into TWO Lithium Heparin (Green-topped) tubes. These samples are referred overnight on Monday – Thursday provided that they are received in the Microbiology laboratory (St. Richard's Hospital, CHICHESTER) before 15:30 on the same day they were taken. Samples received that do not meet these criteria will be unsuitable for analysis and discarded.