

Diagnosing extrapulmonary tuberculosis with the MODS assay

Standard operating procedure for sample preparation and inoculation for TB detection.

Urine

1. SOP version

1.1. Version number

Versión 1.0

1.2. Prepared by

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1.4. Date of approval

25th November 2008

2. Scope of this SOP

This SOP describes the preparation of urine samples for inoculation into a MODS plate for TB detection.

3. Related documents

This SOP assumes the reader is familiar with the MODS user guide. This guide provides a full description of the methodology used for preparing a MODS plate for culture and direct drug susceptibility testing of sputum samples.

4. References

Moore DAJ, Evans CAW, Gilman RH, Caviedes L, Coronel J, Vivar A, Sanchez E, Piñedo Y, Saravia JC, Salazar C, Oberhelman R, Hollm-Delgado M-G, LaChira D, Escombe AR, Friedland JS. *Microscopic observation drug susceptibility assay for the diagnosis of TB*. N Engl J Med 2006; 355 (15): 1539-1550.

5. General comments

This document has been prepared based upon our experience working on detection of TB in urine samples. The bacillary load in urine samples is usually low and that is why we advise performance of only a detection test and not a direct DST. Through this approach positive cultures, which generally appear within 2 weeks, can be harvested for indirect DST more quickly than from conventional cultures.

6. SOP

6.1 General sample considerations

1. The sample should be collected following prior external washing with water.
2. Collect the second squirt of the first morning urine sample directly into a clean wide mouthed bottle.
3. The sample should be sent immediately to the laboratory to be processed; acidic pH and bacterial overgrowth can affect the recovery of bacilli.
4. The sample can be sent with a cold chain at 2-8°C.
5. The sample can be stored refrigerated at 2-8°C preferably for no more than 12 hours.

6.2 Sample required for the process

Collect a minimum of 50ml of urine sample.

6.3 Procedure

1. Concentrate the whole urine sample by centrifugation at 3000g for 15 minutes
2. Carefully pour off supernatant and then collect 2ml of pellet in a 15ml centrifuge tube.
3. Decontaminate the sample following the NaOH-NALC method described for sputum samples. NALC-NaOH exposure time should not exceed 15 minutes.
4. Using a Pasteur pipette resuspend the pellet in a total 2ml of 7H9-OADC-PANTA (from the tube containing 5.1ml 7H9-OADC-PANTA) in the centrifuge tube; mix well.
5. Prepare a smear adding 2 drop (100 μ l) in a slide to perform a Ziehl Neelsen stain.
6. Add the sample suspension to the remaining 7H9-OADC-PANTA; mix well.
7. This is the final sample suspension ready for plating.

6.4 Final MODS plate preparation (detection)

1. Place 1ml of the final sample suspension into each of the 4 wells of a single column in the 24-well plate.
2. Store the remaining sample suspension (1.1ml) into a sterile microcentrifuge tube at 2-8°C as a backup.
3. Repeat with the additional samples until all columns of the plate, except column 3, are filled (or until all samples are plated).
4. Place 1ml of 7H9-OADC-PANTA medium **without sample** in the 4 wells of Column 3 of each sample plate (negative internal controls).
5. Close the plate with its lid and place in a sealable polythene (Zip Plock) bag and seal (bag is not opened again from this point onwards).
6. Incubate at 37°C (CO₂ enrichment is not necessary).

6.5 Plate reading and interpretation

A positive result is defined as two or more colony forming units (≥ 2 cfu) in one or more wells. For more detail on results and reading see the MODS sputum guide.

