

Diagnosing extrapulmonary tuberculosis with the MODS assay

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

Stool

1. SOP version

1.1. Version number

Version 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 stool sample 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

Yajko D, Nassos P, Sanders C, Gonzales P, Reingold A, Horsburgh R, Hopewell P, Chin D and Hadley K. Comparison of four decontamination methods for recovery of *Mycobacterium avium* complex from Stool. *J Clin Microbiol* 1993; 31(2): 302-6.

Oberhelman R, Soto-Castellares G, Caviedes L, Castillo M, Kissinger P, Moore DAJ, Evans C and Gilman RH. Improved recovery of *Mycobacterium tuberculosis* from children using the microscopic observation drug susceptibility method. *Pediatrics* 2006; 118: 100-106.

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 stool samples. Stool is considered as an alternative sample that may provide the opportunity to diagnosis pulmonary tuberculosis in those patients without sputum production. The bacillary load in stool samples is usually low, thus it is advised that only a detection test is performed and not 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. Collect the stool sample in a wide mouth bottle with screw top
2. The sample should be sent immediately to the laboratory to be processed.

3. The sample can be stored refrigerated at 2-8°C preferably for no more than 12 hours; otherwise mycobacteria viability may be affected by overgrowth of stool microflora

6.2 Sample required for the process.

Collect a minimum of 1 gram of stool sample.

6.3 Decontamination Procedure

1. Weight 0.1 gram of stool sample and place into a 15 ml centrifuge tube containing 6ml of sterile distilled water.
2. Cap tube tightly and vortex for 30 seconds; to dissolve the stool sample.
3. Let stand for 15 minutes to allow undissolved particulate stool to separate.
4. Using a Pasteur pipette, transfer 2ml of the supernatant to another sterile 15ml centrifuge tube and proceed to decontaminate the stool suspension follow the NALC-NaOH method described for the sputum sample.
5. After getting the sample pellet, resuspend it in a total of 2ml 7H9-OADC-PANTA (from the tube containing 5.1ml 7H9-OADC-PANTA) in the centrifuge tube with a Pasteur pipette; mix well.
6. Prepare a smear adding 2 drops (100µl) in a slide to perform a Ziehl Neelsen stain.
7. Add the sample suspension to the tube with the remaining 7H9-OADC-PANTA; mix well.
8. 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 details on results and reading see the MODS sputum guide.



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