

Diagnosing extrapulmonary tuberculosis with the MODS assay

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

Nasopharyngeal (NPAs) and Nasogastric aspirate (NGAs)

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 nasopharyngeal and gastric aspirate 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

Oberhelman R, Soto-Castellares G, Caviedes L, Castillo M, Kissinger P, Moore DAJ, Evans C, 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 nasopharyngeal and gastric aspirate samples. These samples are considered as an alternative for the diagnosis of pulmonary tuberculosis in those patients without sputum production. The bacillary load in nasopharyngeal and gastric aspirate samples is usually low and that is why we advise the 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 NPA sample is collected aseptically by insertion of a soft flexible sterile nasopharyngeal tube into the nasopharynx and lavage with 5ml of saline follow by aspiration of respiratory secretions into a sterile container with an electrical suction device.

2. The NGA fluid sample is collected by nasogastric intubation. The sample is augmented by injection of 5 ml of sterile water and then it is aspirated into a sterile container with an electrical suction device.
3. Send the samples immediately to the laboratory maintaining a cold chain (2-8°C) until processing.
4. The sample can be stored refrigerated at 2-8°C preferably for no more than 12 hours.

6.2 Sample required for the process

1. A sample volume of 5ml is recommended.
2. Concentration is required to obtain a 2ml sample. Place the sample into a 15ml centrifuge tube and concentrate by centrifugation at 3000g for 15 minutes.
3. Using a sterile Pasteur pipette discard the supernatant to leave a total volume of 2ml of concentrated sample.

6.3 Procedure

1. Decontaminate the 2ml of sample following the NaOH-NALC method as described for sputum samples. NaOH-NALC exposure time should not exceed 15 minutes.
2. Using a sterile Pasteur pipette resuspend the pellet in a total of 2ml 7H9-OADC-PANTA (from the tube containing 5.1ml 7H9-OADC-PANTA) in the centrifuge tube; mix well.
3. Prepare a smear adding 2 drop (100µl) in a slide to make a Ziehl Neelsen stain.
4. Add the sample suspension to the tube with the remaining 7H9-OADC-PANTA; mix well.
5. 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** into 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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