Diagnosing extrapulmonary tuberculosis with the MODS assay

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

Lymph node aspirate

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 a lymph node aspirate 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

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 lymph node aspirate samples. The bacillary load in these samples could be low; if this is the case we advise that only a detection test is performed and not a direct DST; if the sample is smear-positive then it is possible to perform a direct DST. If only culture is performed 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. Lymph node aspirate samples are aseptically taken by medical personal and collected in a sterile container containing 2-3ml sterile saline at 0.85%, without preservative.
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 24 hours.

6.2 Sample required for the process

Collect a minimum of 2-3ml of lymph node aspirate sample

6.3 Procedure

Split the sample volume in sterile centrifuge tubes.

- 1ml is used for direct inoculation
- 2ml is used for decontamination by the NaOH-NALC method as per sputum sample procedure.

6.3.1 Direct process

1. Using a sterile Pasteur pipette, resuspend 1ml of the lymph node aspirate with 2ml of 7H9-OADC-PANTA (from the tube containing 5.1 ml 7H9-OADC-PANTA) in a tube; mix well.
2. Prepare smear adding 2 drops (100µl) in a slide to perform a Ziehl Neelsen stain.
3. Add the sample suspension to the tube with the remaining 7H9-OADC-PANTA; mix well.
4. This is the final sample suspension ready for plating.

6.3.2 Decontamination process

1. Decontaminate 2ml of the sample following the NaOH-NALC method described for the sputum sample. 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 5.1 ml 7H9-OADC-PANTA) in the centrifuge tube; mix well.
3. Prepare a smear adding 2 drop (100µl) in a slide to perform 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 (2.1 or 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 (CO_2 enrichment is not necessary).

Note

Decontaminated and direct samples are inoculated in separate columns (4 wells for each sample), but can be plated on the same plate.

For direct DST the volume to inoculated into each well should be as per MODS sputum guide (see item 7.5 MODS sputum guide)

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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David Moore November 2008