

Diagnosing extrapulmonary tuberculosis with the MODS assay

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

Pleural, synovial and pericardial fluids

1. *SOP version*

1.1. Version number

Version 1.0

1.2. Prepared by

Dr. David Moore

Laboratorio de Investigación de Enfermedades Infecciosas.

Universidad Peruana Cayetano Heredia, Lima, Perú.

1.3. Approved by

Candidate Msc. Luz Caviedes

Lic.T.M Jorge Coronel

Tec. Pilar Navarro

Laboratorio de Investigación de Enfermedades Infecciosas.

Universidad Peruana Cayetano Heredia, Lima, Perú

1.4. Date of approval

25th November 2008

2. *Scope of this SOP*

This SOP describes the preparation of serous fluid 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

Tovar M, Siedner MJ, Gilman RH, Santillan C, Caviedes L, Valencia T, Jave O, Escombe AR, Moore DAJ, Evans CA. *Improved diagnosis of pleural tuberculosis using the microscopic observation drug susceptibility technique*. Clin Infect Dis 2008; 46: 909-12

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

The diagnosis of pleural tuberculosis is best made by a combination of diagnostic tests. Culture of pleural fluid may be helpful, as may estimation of pleural fluid adenosine deaminase (ADA) concentration and pleural fluid cytology. However, culture of pleural tissue obtained by biopsy combined with histological examination provides the most sensitive combined approach. Smear microscopy of pleural fluid is rarely useful; smear microscopy of biopsy material is occasionally informative. Decontamination of pleural fluid samples reduces sensitivity of MODS culture without significantly affecting culture contamination rate and is thus not recommended.

To our knowledge there are no published data comparing the performance of MODS with other diagnostic methodologies for detection of *Mycobacterium tuberculosis* in pericardial fluid and synovial fluids. Decontamination of fluid samples is not considered necessary prior to culture however when it is suspected that the sample is not sterile, it may be divided into two equal volumes, one for inoculation after decontamination and the other for direct inoculation.

6. SOP

6.1. General sample considerations

1. Fluid samples are taken from normally sterile affected site.
2. The samples are collected in a variety of sterile container without preservative.
3. The sample should be sent immediately to the laboratory to be processed.
4. The sample can be stored refrigerated at 2-8°C preferably for no more than 24 hours.

6.2. Sample required for the process

A sample volume of 5ml is recommended. Concentration of the sample is required.

6.3. Procedure

6.3.1. Direct process (Sterile sample)

1. Sample is concentrated by centrifugation at 3000g for 15 minutes.
2. After centrifugation the supernatant is discarded carefully and the pellet is resuspended in a total volume of 2ml 7H9-OADC-PANTA (from the 5.1ml of 7H9-OADC-PANTA); mix well using a sterile Pasteur pipette.
3. Prepare a smear adding 2 drops (100µl) in a slide to perform a Ziehl Neelsen stain.
4. Add the sample suspension into the tube with the remaining 7H9-OADC-PANTA; mix well
5. This is the final sample suspension ready for plating.

Notes:

If it is suspected that the fluid sample is slightly contaminated and the culture will likely get contaminated, it may be processed by direct inoculation in media 7H9-OADC with 2X PANTA (double the concentration of PANTA); however if the sample is deemed significantly contaminated then it should proceed to full decontamination.

7H9-OADC-2X PANTA medium can be used to reduce the probability of contamination in fluid samples deemed to be minimally contaminated, however this is not a guarantee of contamination-free culture.

PANTA 2X is obtained by adding 200µl of PANTA instead of 100µl.

6.3.2. Decontamination process

1. Sample is concentrated by centrifugation at 3000g for 15 minutes.
2. After centrifugation the supernatant is carefully removed, leaving a volume of about 2ml.
3. Decontaminate the 2ml sample following the NaOH-NALC method described for sputum samples. NaOH-NALC exposure time should not exceed 15 minutes.
4. 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.
5. Prepare a smear adding 2 drop (100µl) in a slide to make a Ziehl Neelsen stain.
6. Add the sample suspension into the tube to the remaining 7H9-OADC-PANTA; mix well.
7. This sample suspension is now ready for plating.

6.4. Final MODS plate preparation

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).

Notes

If the sample is processed by both direct inoculation and inoculation after the decontamination procedure, the decontaminate and direct sample should be inoculated in separate columns (4 wells for each sample), and can be inoculated into the same plate.

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.



David Moore

November 2008