

Diagnosing extrapulmonary tuberculosis with the MODS assay

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

Cerebrospinal fluid (CSF)

1. SOP version

1.1. Version number

Version 1.0

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1.4. Date of approval

25th November 2008

2. Scope of this SOP

This SOP describes the preparation of cerebrospinal 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. This document should also be read in conjunction with the document “M Caws Vietnam CSF SOP” which details the whole CSF MODS culture procedure (not just sample preparation) as performed in Vietnam.

4. References

Caws M, Thi Minh Ha D, Torok E, Campbell J, Dang Anh Thu D, Thi Hong Chau T, van Vinh Chau N, Tran Chinh N, Farrar J. *Evaluation of the MODS culture technique for the diagnosis of tuberculous meningitis*. PloS ONE 2007; 2 (11): e1173.

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 the SOP developed by Maxine Caws in Vietnam. The Vietnam laboratory is one of the leading diagnostic laboratories for tuberculous meningitis in the world and undoubtedly has the greatest experience with MODS for this indication. Maxine comments in her laboratory SOP “Direct drug susceptibility testing in MODS is not yet validated for CSF samples – the low colony numbers in the drug-free wells may preclude reading of the drug-containing wells. For this reason in Vietnam, the site with the greatest experience of CSF MODS culture, this method is used for detection alone and not for DST. Through this approach positive cultures, which generally appear within 2 weeks, can be

harvested for indirect DST more quickly than from conventional cultures. Furthermore, in Vietnam 48 well plates are used rather than 24 well plates."

Inoculating the concentrated CSF sample into a single well delivers better sensitivity than dividing the sample into several wells.

6. SOP

6.1. General sample considerations

1. Cerebrospinal fluid (CSF) samples are taken from a normally sterile site (the meningeal space).
2. The largest volume of CSF which can be safely obtained should be collected in a sterile tube.
3. Addition of anticoagulant to the sample is not necessary
4. The sample should be sent immediately to the laboratory to be processed
5. The sample may be stored refrigerated at 2-8°C preferably for no more than 12 hours.

6.2. Sample required for the process

1. Volumes of at least 5ml are desirable for high sensitivity (>60%) in microbiological diagnosis of tuberculous meningitis.
2. Concentrate the entire sample by centrifugation.

6.3. Procedure

1. Place the sample into a 15ml centrifuge tube and concentrate at 3000g for 15 minutes.
2. Carefully remove the supernatant to leave approximately 350µl of pellet, using a sterile Pasteur pipette.
3. Using a Pasteur pipette resuspend the pellet and prepare a smear adding 2 drops (100µl) to a slide to make a Ziehl Neelsen stain.
4. The remaining sample suspension is ready for plating.

6.4. Final MODS plate preparation (detection only)

Using a Pasteur pipette 5 drops (250µl) of the final sample suspension are removed to inoculate into a MODS well containing 750µl of 7H9-OADC-PANTA.

6.5. Procedure if CSF sample is bloody

1. If CSF sample is sufficiently bloodstained that it will interfere with plate reading, red blood cells should be lysed prior to inoculation into a MODS plate.
2. Lysis procedure
 - Add 1000 μ l of sterile distilled water to the sample
 - Vortex and leave for 10 minutes
 - Centrifuge at 13,200g for 2 minutes
 - Remove 1000 μ l supernatant and discard.

6.6. Plate reading and interpretation

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



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