

## **Diagnosing extrapulmonary tuberculosis with the MODS assay**

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

#### **Tissue biopsy**

##### **1. *SOP version***

###### **1.1. Version number**

Version 1.0

###### **1.2. Prepared by**

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###### **1.4. Date of approval**

**25<sup>th</sup> November 2008**

##### **2. *Scope of this SOP***

This SOP describes the preparation of a tissue biopsy 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**

Tovar M, Siedner MJ, Gilman RH, Santillans 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**

Data comparing MODS with other culture methodologies for detection of *Mycobacterium tuberculosis* from biopsy specimens is lacking, with the exception of pleural biopsy material. Decontamination of biopsy samples taken from sterile sites may reduce sensitivity of MODS culture without significantly affecting incidence of culture contamination rate and is thus no longer recommended. However, the procedure is described below in case laboratories wish to use it.

## **6. SOP**

### **6.1 General sample considerations**

1. Tissue biopsy samples are aseptically taken by medical personal in a sterile container containing 2-3ml sterile saline to 0.85%, without preservatives.
2. The sample should be send immediately to the laboratory to its process.
3. The sample can be preserve in refrigeration at 2-8°C preferably for no more than 24 hours.

## 6.2 Sample required for the process

Collect a minimum of 5mm<sup>3</sup> of biopsy sample.

## 6.3 Procedure.

1. The tissue sample is homogenized in 300-400µl of sterile saline with a sterile glass mortar (type Potter-Elvehjem).
2. A sample volume of 4ml is required after the tissue has been homogenized.
3. Split the sample into two sterile centrifuge tubes:
  - 2ml is used for direct inoculation.
  - 2ml is decontaminated using the NaOH-NALC method as per sputum sample.

### 6.3.1 Direct process.

1. Using a sterile Pasteur pipette, resuspend 2ml of the homogenized sample with 2ml 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 homogenized 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 volume of 2ml 7H9-OADC-PANTA (from the tube containing 5.1 ml 7H9-OADC-PANTA) in a 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 only)

Inoculate an aliquot of the non-decontaminated homogenate and decontaminated samples as follows:

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) in 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<sub>2</sub> enrichment is not necessary).

### Notes

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

### 6.5 Plate reading and interpretation

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



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