



# MycoDot™

## For the Detection of Antimycobacterial Antibodies as an Aid in the Diagnosis of Active Tuberculosis

### Background

Tuberculosis (TB) has been a cause of significant morbidity and mortality for mankind throughout history (1). Currently, there are 20 million cases of TB worldwide with 8 million new cases each year. Three million deaths annually are directly attributable to tuberculosis (2,3). TB continues to be a significant problem in the developing world. In addition, there has been a dramatic increase over the last five years in the incidence of tuberculosis in developed countries. This increase can be attributed to increases in the incidence of Acquired Immunodeficiency Syndrome (AIDS) (3), immigration, and the other primary risk factors for the disease such as poverty, malnutrition and overcrowded living conditions (8). Tuberculosis usually presents itself as an infection of the lungs but it can affect virtually any organ system in the body (1,3). It is caused by the bacterium *Mycobacterium tuberculosis* and is primarily spread by bacteria present in aerosols generated by coughing. Since TB is curable with the use of antibiotics, early diagnosis will dramatically reduce patient morbidity and mortality if diagnosis is followed by appropriate intervention.

Currently, the diagnosis of pulmonary TB is based largely on the microscopic detection of the causative organism in patient sputum, pleural fluid, and bronchoalveolar lavage. This method of detection, the acid fast bacillus (AFB) smear, is error prone. It has a sensitivity of, at best, 20-40% in developing countries and 40-60% in developed countries. Culturing the bacteria on solid media yields significantly better results. However, this procedure is much more expensive and, due to the slow rate of growth of the bacteria, results are seldom obtained before 4 weeks. Detection of bacteria by AFB smear or culture is only possible for bacillary cases of pulmonary TB (when bacteria are present in the body fluids) and for each case of pulmonary TB that is bacillary, there are 2-3 that are non-bacillary (bacteria are not detected in the specimen by either of these two methods). While the chest X-ray is used in the diagnosis of pulmonary TB, it is often inadequate due to the non-specific nature of the findings (4). Also, the new problem of HIV-infected TB patients has created a complication in the use of this method, since HIV infection can change the classical appearance of the chest X-ray (8,9). In addition, an appreciable number of all

TB cases are extrapulmonary, for which diagnosis is made on the basis of clinical presentation and detection of the bacteria in biopsy specimens. For these reasons, a test capable of detecting active TB irrespective of its focus of infection in the body would be a valuable addition to effective diagnosis. Mossman Associates' MycoDot test meets this need.

### Test Description

MycoDot is a simple, rapid (20 minutes) and reliable test that can detect antimycobacterial antibodies in serum or blood. The test offers a low cost, single visit aid in the diagnosis of tuberculosis with good sensitivity and-excellent specificity. A positive reaction will only occur in patients with active mycobacterial diseases like TB. Healthy, infected (PPD-positive) patients and/or BCG-vaccinated individuals react negatively.

The MycoDot test employs lipoarabinomannan (LAM) antigen, bound to plastic combs. When the combs are incubated in diluted serum/blood, specific anti-LAM antibodies from the sample, if present, bind to the antigen. The combs are then washed to remove non-specific antibody, and incubated in a suspension of colored particles which bind to the bound anti-LAM antibodies. If enough of the specific antibodies are present in the serum sample, a colored spot will form where the antigen is attached to the plastic comb. The sensitivity of the test is calibrated so that only cases of active mycobacterial disease such as tuberculosis will generate a colored spot which is as strong or stronger than the weakest positive spot on the reference comb that is provided as a guide to interpret results.

### Sample Preparation

Whole blood, serum or heparin derived plasma can be used. **Plasma derived by the addition of divalent cation chelators such as sodium citrate or EDTA must not be used.** Specimens can be kept at 2-8°C for short-term storage. However, they must be stored frozen (-20°C or lower) for long-term storage. It is recommended that whole or heparinized blood be centrifuged to remove red blood cells, prior to freezing. Grossly hemolyzed and/or contaminated samples must not be used.

## Materials provided

The kit components for tests include

- Twelve antigen coated combs
- One dropper bottle of Signal generating reagent
- One bottle of 5X Concentrated rinse buffer
- One dropper bottle of sample diluent
- One bottle of negative control serum
- One bottle of positive control serum
- One reference comb
- One wash tray
- Two microtiter test plate(s)

## Materials required but not provided

- Pipette and disposable tips capable of delivering 40 to 80 microliters ( $\mu$ ls)
- 100 ml graduated cylinder
- Distilled Water
- Timer capable of timing 6 and 10 minutes
- Paper towels or other absorbent pad

## Storage

When stored between 2-8°C, all kit components are stable until the expiration date shown on the label. The pouch containing the antigen coated combs should be brought to room temperature before opening to prevent condensation. Unused antigen combs should be stored in the aluminum pouches along with the silica gel bag and tightly closed in the zipper seal bag, to protect from moisture during storage. Once diluted, the rinse buffer is stable for one week if stored between 2-8°C.

**Warning: All samples for analysis should be considered potentially infectious and handled with care. Positive and negative control sera have been serotested for HIV and HBsAg and are negative. They have also been filtered through a 0.2  $\mu$ m filter to remove bacteria. Nevertheless, all samples should be handled with appropriate precautions.**

## Precautions

1. Upon assay completion, collect remnants of samples, buffers and aspirated reagents to be disposed in a container specifically kept for this purpose and autoclave for 1 hour at 121°C or treat with sodium hypochlorite (10% final concentration) for 30 minutes.
2. Wear gloves while performing the test and wash hands thoroughly after completion of test.
3. Do not smoke, eat or drink in areas where biological samples are handled.
4. Avoid splashing or spilling of reagents.
5. Extreme care should be taken to avoid microbial contamination of reagents.

6. In one test run, do not combine antigen combs and signal generating reagent from boxes with different lot numbers.
7. Use only microtiter plate wells which have not been previously used.
8. Do not let diluted serum sit in microplate wells for longer than twenty minutes without testing.

## Limitations

Since the test detects antimycobacterial IgG antibodies, patients who have had active TB in the past may continue to have antibody titers above the cut-off after they have been cured of TB. The attending physician should therefore interview the patient regarding any history of tuberculosis.

If a TB patient is immunocompromised with lowered CD4 counts, such as in certain AIDS patients, the MycoDot test may not provide a positive result since antimycobacterial antibodies may not be present at levels indicative of active disease.

A positive reaction may be obtained with MycoDot for active mycobacterial diseases other than TB. However, in general, non-tuberculous active mycobacterioses seldom occur except when the immune system is compromised; in those circumstances the antibody response is curtailed and the MycoDot reaction is likely to be negative. Leprosy patients without TB will react positively by the MycoDot, however; the clinical presentation of leprosy cannot be confused with that for TB. Therefore, for all practical purposes, when a patient presents with symptoms suggestive of TB, a positive reaction by MycoDot, is an indicator of active tuberculosis.

## Set Up for the Test

Dilute the concentrated rinse buffer 1:5 with distilled water. Add 1 2mL of the concentrated rinse buffer to 48 ml of water. Fill the wash tray with the rinse buffer taking care to avoid foaming when pouring. Use the resulting 60 ml of rinse buffer to wash no more than 2 combs. **Bring all samples and kit components to room temperature prior to testing.**

## Test Procedure

1. While holding the dropper bottle at a 45° angle, deliver 5 drops of sample diluent to each well of alternating 8 well columns (1,3,5,7,9 and 11) on the microtiter plate for each sample to be tested as well as for the positive and negative control sera. Positive and negative assay controls, supplied in the kit, are to be routinely tested each day the test is performed, or as lab protocol dictates.
2. While holding the dropper bottle at a 45° angle, deliver 4 drops of signal generating reagent to the corresponding wells in adjacent columns (2,4,6,8,10 and 12) on the plate for each sample/control serum to be tested.

3. Add 20  $\mu\text{l}$  of serum or plasma (or 35  $\mu\text{l}$  of heparinized or whole blood) to each sample diluent well. Mix thoroughly by pipetting back and forth in the well.
4. Remove a test comb from a foil pouch and label it on the dull side in the space above the teeth with the appropriate test sample number for each sample/control to be tested.
5. Set a timer to 6 minutes, and holding the comb vertically with the teeth pointing down, place the comb, dull side facing the user, into the first row of diluted samples and begin timing. Gently rock the comb back and forth 8–10 times. Repeat the rocking step at least one more time approximately halfway through the incubation step.
6. After 6 minutes, remove the comb and touch the tips of the teeth to a paper towel to remove excess liquid. Blot only the tips of the teeth. Rinse the teeth of the comb in the diluted rinse buffer by moving the comb back and forth across the wash tray 10 times. Again, touch the tips of the teeth to the paper towel to remove excess liquid.
7. Set the timer to 10 minutes, place the teeth of the comb into the signal generating reagent and begin timing. Gently rock the comb back and forth 8-10 times.
8. At the end of 10 minutes, remove the comb and blot and rinse as in step 6.

**Note: The diluted rinse buffer in the tray must be changed for every two combs that have been washed.**

9. Touch off excess liquid and allow the comb to air dry. To determine the result of each sample, place the comb on a flat solid surface with a white background under good lighting and read the reaction perpendicular to the comb. Incandescent light may make it difficult to interpret faintly positive reactions. Compare the intensities of the test reactions to those on the reference comb provided with each kit. Note: Too much light on the comb or viewing the comb at an angle can make the interpretation of results difficult. Placing the comb against a white background allows for easier interpretation.
10. Record the results as positive (+) or negative (–)
11. Once dried, the comb may be attached with transparent tape to a patient file or notebook to serve as a permanent record.

### Interpretation of Results

To interpret the reaction generated by the MycoDot test, one must use the reference comb provided in the kit. A colored spot as intense, or more intense, than the weakest positive spot on the reference comb is to be considered a positive reaction. A spot less intense than the weakest positive spot on the reference comb, or no spot at all, is a negative reaction. For borderline reactions, it is recommended that a fresh sample be drawn after 2-4 weeks and that it be retested. During this period, the attending physician should obtain as

much clinical information as possible to aid in making the diagnosis.

### Performance Characteristics

For the MycoDot test, a total of 1,602 samples were evaluated in three countries. Of the 564 pulmonary TB patients tested, 396 reacted positively in the MycoDot test for an overall sensitivity of 70.2%. Of the 564 pulmonary TB samples, 328 were tested for the presence of anti-HIV antibodies. Of 28 HIV-positive TB patients, 21 reacted positively in the MycoDot test for a sensitivity of 75% in this population. Of the 1,038 TB negative individuals tested, 51 reacted positively, for an overall specificity of 95.1%. It should be noted that among the false positive reactors, active TB or other active mycobacterial disease could not be conclusively ruled out. There were 120 patients in the negative control group that were directly under the care of a physician involved in the evaluation of the MycoDot test, and in his clinical judgment, these patients did not have tuberculosis. Only four patients in this group reacted as false positive, for a specificity of 96.7%. Among the pulmonary TB negative group 182 had been vaccinated with the BCG Vaccine. In this group only 5 reacted as false positive for a specificity in this population of 97.3%.

In general, the diagnosis of TB was made by a combination of AFB smear examination result, culture result, and/or a combination of chest X-ray finding and clinical symptomology. In some tuberculosis patients with active disease, antimycobacterial antibodies are not detectable and these individuals will react as false negative. There is some evidence that the presence of circulating antigen-antibody complexes may reduce the detectable antibody titer (15).

It should be noted that the results of the MycoDot test must be evaluated in light of all available clinical information including results of AFB smear, chest X-ray, and clinical symptoms such as fever, malaise, weight loss, and cough.

Precision studies of the MycoDot test were performed as follows. Eight serum samples were tested a total of nineteen times each, by four different analysts on six different days. Of the eight specimens, there were three moderate to strong reactors, four were borderline positive reactors, and one was negative. Except for one borderline positive reactor, all of the samples were tested and interpreted with one hundred percent accuracy by each of the four analysts. The borderline sample was interpreted differently by the same analysts on different occasions. In addition, four samples were tested by the MycoDot test, sixteen times each, by one analyst. The results for each of the four samples was identical for every determination.

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