Destinatari: Personale del Settore Diagnosi Microbiologica Avanzata e Settore Ricerca Micobatteri

CONTENT

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1. SCOPE

According to the World Health Organization recommendations, it is imperative that all mycobacteria isolates be identified at least to the level of *M. tuberculosis complex* vs. non-tuberculous mycobacteria (NTM) and that a rapid and affordable method of species identification be used.

2. APPLICATION

This IOS describes how to use rapid immunochromatographic identification tests for the identification of *M. tuberculosis complex* isolates and it is applicable to the whole mycobacteria area. For the identification of Mycobacterium species belonging to *M. tuberculosis complex* species or mycobacteria other than tuberculosis by chemical assays, refer to the IOS EBP-TM 002 “LiPA Assays” and IOS EBP-DMA 009 “Test Biochimici e Colturali per l’Identificazione dei MNT”.

3. DEFINITIONS AND ABBREVIATIONS

NTM	Non Tubercle Mycobacteria
MNT	Micobatteri Non Tubercolari
BSC	Biological Safety Cabinet
TB	Tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
TBc	Tuberculosis complex

4. RESPONSIBILITIES

The supervision and the correct application of the following instruction is the responsibility of the area coordinator. The execution of the test is responsibility of area technicians, master students and coordinator.

5. EQUIPMENT AND MATERIALS

- BSC: Biological Safety Cabinet
- BD MGIT™ TBc Identification Test kit
- Capilia TB-Neo kit
- SD BIOLINE TB Ag MPT64 Rapid Test kit
- Sterile loops
- Sterile pipette tips
- Timer
- Eppendorf tubes
- Pipettes 100 µl; 1000 µl
- Sterile water

6. PROCEDURES

6.1 Capilia TB-Neo (TAUNS Laboratories, Inc.)

The Capilia TB-Neo kit adopts an immunochromatography method, which can detect the MPB64 antigens specifically produced by the *M. tuberculosis complex*. Thanks to this method, the kit is able not only to detect an *M.tuberculosis complex* in bacterial isolates specifically with high sensitivity but also to perform rapid tests with only a simple operation without special instruments and equipment.
This product is intended to detect MPB64 (mycobacterial protein fraction from BCG of Rm 0.64 in electrophoresis), a protein secreted from the cells during the culture of an *M.tuberculosis* complex. Non-tubercle mycobacteria (NTM) produces no MPB64. Moreover, MPB64 is a protein specifically secreted by the member of *M.tuberculosis complex* (*M.tuberculosis, M.bovis, M. africanum and M. microti).*

Test procedure

This product is a test plate that consists of a carrier strip composed of a sample placing area, a reagent area containing a colloidal gold-labeled anti-MPB64 monoclonal antibody (mouse) and a developing area where the anti-MPB64 monoclonal antibody (mouse) and an anti-mouse immunoglobulin polyclonal antibody (rabbit) are fixed.
Mycobacterial isolates are tested only when in presence of pure cultures (liquid and solid positive cultures) confirmed by AFB staining demonstrating chords formations (for liquid cultures)

If the isolate is on solid cultures:

1. Dispense 0.2mL of extraction buffer into a tube. Separately sold.
2. Pick 1μL of bacteria (equivalent to the amount of a 1mm-diameter micro-loop) from the bacterial colony that has grown on the solid medium.
3. Suspend the collected bacteria in the buffer solution in the tube.
4. Close the tube with a stopper and fully suspend with a vortex mixer. Then, use the bacterial suspension as specimens:

- drop an 80～100μL specimen into the sample placing area of the test plate.
- Observe the reading area of the test plate after 15 min and interpret the result as follows:

- **Positive**, if a red purple line is observed in the reading areas of both [T] and [C]
- **Negative**, if a red purple line is not observed in the reading area [T] but the color is observed in the reading area [C]

Test results must be interpreted within 60 min after placing each specimen on the test plate because the results may change for some plates due to dryness occurring after the elapse of time.

If the isolate is in liquid culture (BBL MGIT 7-ml tube):

1. Drop 80-100 μL positive culture directly into the sample placing area and wait 15 minutes for results

6.2 BD MGIT TBc Identification Test

The TBc ID test is a chromatographic immunoassay for the qualitative detection of *M.tuberculosis complex* from an AFB smear-positive BBL MGIT tube. This product detects MPT64, a mycobacterial protein fraction that is secreted from *M.tb* cells during culture. When samples are added to the test device, MPT64 antigen binds to anti MPT64 antibodies conjugated to visualizing particles on the test strip. The antigen-conjugate complex migrates across the test strip to the reaction area and is captured by a second specific MPT64 antibody applied to the membrane.
If the MPT64 antigen is present in the sample, a color reaction is produced by the labeled colloidal gold particles and is visualized as a pink to red line.

- **Sample collection and preparation**

This test is designed to identify MTbc from AFB smear-positive tubes (4 ml and 7ml).
The presence of AFB in a positive MGIT tube should be confirmed using an AFB smear prior to conducting the test.
Positive MGIT tube can be stored at 2-37℃ for up to 10 days after MGIT tube positivity and prior to testing with the TBc ID device. If necessary, positive MGIT tube may be stored and maintained at -20 to 8℃ for up to two months.
-AFB smear-positive MGIT tubes can be tested in the TBc ID device within 10 days after MGIT tube positivity.
-If device are refrigerated, they must be brought to ambient temperature in the foil pouch prior to testing.

1. Remove the TBc ID device from its foil pouch immediately before testing. Place the device on a flat surface.
2. Label one device for each sample to be tested.
3. Thoroughly mix the sample (AFB smear-positive MGIT tube) by inverting or vortexing. Do not centrifuge.
4. Remove cap from MGIT tube and using a sterile pipette tip, pipette 100 µl of the sample well (as indicate by the teardrop) of the appropriately labeled device. Tightly replace cap on MGIT tube. Start timer for 15 min.
5. Read result at 15 min and record test result. Do not interpret test after 60 min.

The present assay has not been validated for isolates on solid cultures yet.

- **Quality Control**

Each device contains both positive and negative internal/procedural controls.
The appearance of a control line in the read window at the Control “C” position provides an internal positive control that validates the proper reagent function and assures the correct test procedure was followed. The membrane area surrounding the test and control line is the internal negative control for the device. A background area that is white to light pink indicates that the test is performing correctly.

- **Interpretation of result**

Positive test for TBc (MPT64 antigen present): a pink to red line appears at the test “T” position and the control “C” position in the read window. This indicates MPT64 antigen was detected in the sample. The intensity of the C and T lines may vary. The background area should be white to light pink.

Negative test for TBc (no MPT64 antigen detected): no pink to red line visible at the test "T" position of the read window. This inidicates that MPT64 antigen was not detected in the sample. A line at the control "C" position read window indicates proper performance of the test procedure. The background area should be white to light pink.

Invalid test: the test is invalid if no pink to red line is visible at the control "C" position in the read window or if the background area color inhibits test interpretation. If invalid, the sample must be retested with a new device.

6.3 SD BIOLINE TB Ag MPT64 Rapid Test

This test is a rapid immunochromatographic identification test for the *M. tuberculosis complex* isolates that uses mouse monoclonal anti-MPT64.
Mouse monoclonal anti-MPT64 were immobilized on the nitrocellulose membrane as the capture material (test line). Another antibody, which recognized another epitope of MPT64, conjugated with colloidal gold particles were used for antigen capture and detection in a sandwich type assay.

- **Specimen preparation and procedure of the test**

Liquid cultures:
100 µl of sample taken from liquid cultures processed by standard sputum specimen decontamination procedure (IOS EBP-DMA 004) can be applied directly to the sample well without use of the sample preparation procedure.
Solid cultures:
-colonies: for sample preparation from solid culture, 3-4 colonies should be suspended in 200 µl of extraction buffer prior to test.
-condensation fluid: if there is condensation fluid of slant agar tubes, 100 µl of sample taken from condensation fluid can be applied directly to the sample well, or colonies can be suspended in this condensation fluid instead of extraction buffer.

1. Remove the test device from the foil pouch, and place it on a flat, dry surface.
2. Add 100 µl of liquid culture (or 100 µl of suspended solid culture in buffer) into the sample well.
3. As the test begins to work, you will see purple color move across the result window in the center of the test device.
4. Interpret the result in 15 minutes after sample application.

- **Interpretation of the test**

A color band will appear at left section of the result window to show that the test is working properly. This band is the Control Band.
The right section of the test result window indicates the test results. If another color band appears at the right section of the result window, this band is the Test Band.

- **Negative** Result: the presence of only control band within the result window indicates a negative result.
- **Positive** Result: the presence of two color bands within the result window, no matter which band appears first, indicates a positive result.
- **Invalid** Result: if the control band is not visible within the result window after performing the test, the result is considered invalid. It is recommended that the specimen should be re-tested.

7. SAFETY RULES AND PRECAUTIONS

Capilia TB-Neo: Interfering Substances

Clinical tests have been carried out for this product using cultures from sputum, bronchial lavage fluid, hydrothorax, gastric juice and purulent fluid as specimens, and no interference to the test results by clinical samples has so far been observed. In addition, we have used the following media for AFB, and no interference in the test results by the media was observed:

・Egg-based media: 3% Ogawa media, 2% Ogawa media, 1% Ogawa media, Loewenstein-Jensen (LJ) media

・ Agar media: Middle Brook7H10 agar media, Middle Brook7H11 agar media

・ Liquid media: Middle Brook7H9 liquid media, Dubo's liquid media, Kirchner media, Sauton's media

As explained above, no impact from different kinds of clinical samples or culture media has so far been found in the study. However, it is not known whether or not any other substances present in specimens may affect test results.

Capilia TB-Neo: Precautions when interpreting test results

-If no line is seen at [C] in the reading area, there is a possibility of operational problems or deterioration in the quality of the reagent used. Therefore, retesting is required using another test plate.

-The test results may change for some plates due to dryness occurring after a lapse of time. In such a case, retesting is required using another test plate.

-If a test result using this kit is interpreted as positive, the presence of *M.tuberculosis* in the specimen is strongly suggested. However, there is a possibility of combined infections of *M.tuberculosis* and non-tuberculous acid-fast bacteria, and an infection with non-tuberculous acid-fast bacteria is not excludable.

-In the case of protein A-producing strains such as *Staphylococcus aureus*, false positive reactions may occur. Take due precautions for such a case.

-Even if a test result using this kit is interpreted as negative, this may be because it is unable to detect an *M. tuberculosis complex* when the MPB64 concentration in the specimen is below the detection limit or a mutation arises in the MPB64 gene of *M. tuberculosis complex*.

Therefore, a negative result does not necessarily rule out the possibility of infection with *M. tuberculosis.*

BD MGIT TBc Identification Test: Limitations of the procedure

-This test do not rule out the presence of other mycobacterial or mixed bacterial infactions.

-This test is unable to differentiate within *M.tb complex* (MTbc) species.

-This test should not be used solely for the determination of *M.tuberculosis* infection. The test results are to be used in conjunction with information available from the patient's clinical evaluation and other diagnostic procedures.

-A negative result does not always rule out the possibility of infection with *M.tuberculosis*. The device is unable to detect M.tb complex when a mutation arises in the MPT64 gene.

-Some substrains of *M. bovis* BCG among *M. tuberculosis* complex produce no MPT64 antigen and will therefore result in a negative test result with the present device.

SD BIOLINE TB Ag MPT64 Rapid Test: Limitation of the test

-Although the SD BIOLINE TB Ag MPT64 Rapid Test is very accurate in detecting MPT64 antigen, a low incidence of false results can occur.

-Other clinically available tests are required if questionable results are obtained.

-As with all diagnostic test, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by physician after all clinical and laboratory findings have been evaluated.

8. RECORDING AND REPORTING

Results deriving from rapid identification tests must have to be reported into specific databases when required. Rapid identification of *M.tuberculosis complex* is the unavoidable starting point for drug susceptibility testing. Therefore, when not indicated, the presence of DSTs results, implicates previous identification even though not indicated into the database or on the report.

9. RELATED DOCUMENTS

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