IVD

MYCOBACTERIUM TUBERCULOSIS ACID-FAST STAINING SOLUTION (TB STAIN)

Catalog No. 09B0402

3 × 100 mL/3 × 250 mL

INTENDED USE

This assay is based on acid-fast staining technique, intended to apply chemical staining to acid-fast bacteria such as *Mycobacterium tuberculosis*, acting as an important aid in the diagnosis of *Mycobacterium tuberculosis* infections.

INTRODUCTION

Mycobacterium tuberculosis (MTB) is a pathogenic bacterial species in the genus *Mycobacterium* and the pathogen of most cases of tuberculosis (TB)[1].

Globally, the TB disease is an enormous problem. TB is the world's most significant infectious disease from a single infection agent[2].

Hence, the early screening and detection of MTB infection is of great benefit to the control of this disease.

At the moment, the direct microscopic examination of stained sputum smear is still a commonly used method for the diagnosis of tuberculosis, due to its advantages such as fast, convenient and reliable results[3].

BIOLOGICAL PRINCIPLE OF THE PROCEDURE

Acid-fast bacteria such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* possess a lipid coating, hence are difficult to be stained. Once stained, however, the color applied onto them is difficult to fade under acidic alcoholic conditions. Given this property, a special dye is applied to the specimen, then the stained specimen is processed with acid-alcohol. After this step, counterstain is applied which makes the acid-fast bacteria display a red color while other microorganisms and the background display a blue color.

MATERIALS PROVIDED

1. Carbolfuchsin Reagent: a reagent containing basic fuchsin and phenol (1 vial, 100 mL/1 vial, 250 mL)
2. Decolorization Reagent: a reagent containing ethanol and hydrochloric acid (1 vial, 100 mL/ 1 vial, 250 mL)
3. Methylene Blue: (1 vial, 100 mL/ 1 vial, 250 mL)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microscope slides
2. Inoculation loop

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at room temperature and under good ventilation conditions upon receipt. The test kit may be used throughout the expiration date of the kit (24 months from the date of manufacture). Refer to the package label for the expiration date.

SPECIMEN COLLECTION, PREPARATION, TRANSPORT AND STORAGE

1. Specimens collected with right medical techniques such as sputum, urine and pleural effusion may be used.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only. For professional use only.
2. This package insert must be fully understood prior to operation. The operation must be stringently in accordance with the instruction for use.
3. After each use, cap the reagent bottle immediately to avoid vaporization. Once Decolorization Reagent is completed, 3% hydrochloric acid-ethanol solution could be formulated as a supplement.
4. For sputum specimen, the smear should be properly thickened detection rate. For thicker smear, the counterstain time must be controlled. The microscopic examination will be affected if background is too dark.
5. Occasionally the intensity of red stain may be different for the same acid-fast bacterium. Note the difference in red stain intensity when reading.
6. For kit storage, avoid exposure to extreme high or low temperature and sunlight.
7. When acid-fast staining pathologic slides, heating stain method is recommended. For better results, the staining time of Carbolfuchsin should be longer than 5 minutes.
8. Wear disposable gloves when dealing with specimens and reagents. Wash hands after operations. All specimens must be regarded as potentially infectious materials. Waster material must be disposed of safely according to relevant local and national requirements.
9. Components with different lot numbers are not allowed to be exchanged.

ASSAY PROCEDURE

1. Pick the specimens with a inoculation loop. Smear to an ellipse shape with a size of 2.0 × 2.5 cm. Fix the smear with an alcohol lamp.
2. Add Carbolfuchsin Reagent, ensuring the whole smear is covered. Then heat the microscope slide slowly and keep the stain steaming for 5 minutes. Avoid vaporizing to dry or boiling. Add more Carbolfuchsin Reagent when it is about to dry.
3. Wait minutes to cool, rinse with water gently (dry the slide gently with bibulous paper).
4. Decolorize for 2 minutes with Decolorization Reagent until no more red color is coming off. Rinse with water.
5. Rinse with water gently (if red color is still visible on the surface of specimen, add Acid Alcohol again until red is completely decolorized).
6. Apply Methylene Blue for 1 minute. Rinse with water gently and air dry. Bake to dry with an alcohol lamp.
7. Examine the finished slide under a microscope.

INTERPRETATION OF RESULTS

1. Acid-fast Mycobacterium species will appear bright red, while other bacteria and cells display blue.
2. *Mycobacterium tuberculosis* is a rod-shaped (straight or partially curved rod) Gram-positive bacterium, about 1.3 to 3.5 μm long and 0.3 to 0.5 μm wide. It does not form flagella or spores. Mycobacterium can be readily stained by acid-fast stain. Under the microscope, acid-fast bacteria may appear rods in clump, or as particles. At lower cell, certain special shapes, such as "V", "Y" and "T", can be formed via the linkage of 2～3 cells.

LIMITATIONS

1. Some other bacteria such as *Mycobacterium leprae* also display a red color.

SYMBOLS

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
REF	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

1. Kenneth James Ryan, C. George Ray, John C. Sherris *Sherris Medical Microbiology : An Introduction to Infectious Diseases;* McGraw-Hill Professional: 2003;
2. Barry R. Bloom *Tuberculosis: pathogenesis, protection, and control;* ASM Press: 1994;
3. Wu M., Ai Y. *Chinese Journal of Antituberculosis* **2007,** 29, 288.

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