

EZER™ TB MPT64

INTENDED USE

The **EZER™ TB MPT64** antigen rapid test device is an immunochromatographic assay (ICA) using anti-MPT64 monoclonal antibodies for directly qualitative detection of *Mycobacterium tuberculosis* complex (MTBC) antigen. The device will detect the following species of the MTBC: *M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*. The positive result of the test will indicate the presence of MPT64-secretory *M. tuberculosis* Complex in the culture medium. For professional use only.

BACKGROUND

Tuberculosis (TB) is a chronic disease caused by infection with *Mycobacterium tuberculosis*. The number of new TB cases and deaths from TB is estimated at around 10.0 million (2017) and 1.6 million (2017) respectively. There were cases in all countries and age groups, but overall 90% were adults (aged ≥ 15 years), 9% were people living with HIV (72% in Africa) and two thirds were in eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). These and 22 other countries in WHO's list of 30 high TB burden countries accounted for 87% of the world's cases. Only 6% of global cases were in the WHO European Region (3%) and WHO Region of the Americas (3%).

PRINCIPLE

This methodology is intended to detect MPT64 protein secreted from *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*) while culturing and however non-tuberculosis mycobacterium (NTM) produces no MPT64.

The ICA test device for MPT64 consists of a plastic cassette with a sample pad, a reagent pad, a nitrocellulose membrane, and an absorbent pad. On the slide there is a sample well where the sample is applied and two test reaction zones where the results of the test and control are observed as pink to reddish purple bands. A 100 μ L sample is applied to the test well, and it flows through the nitrocellulose membrane laterally. The monoclonal antibodies against MPT64, which is conjugated with colloidal gold and is immobilized at the membrane, is rehydrated by the liquid from the sample and reacts with the MPT64 antigen if present in the sample. The MPT64-antibody complex then flows further laterally and is captured by the test band,

where anti-MPT64 antibody is present, producing a color band. A second anti-mouse immunoglobulin G antibody immobilized at the control band reacts with the free gold conjugate antibody when the sample continues to migrate through the control band area and gives another reddish-purple color band. The test is read at 15 min or up to 60 min after sample added. If the test band is positive, it indicates the presence of the *M. tuberculosis* complex. The control band color indicates that all of the test reactions performed satisfactorily and should be positive in every testing.

WARNINGS and PRECAUTIONS

1. For *in vitro* Diagnostic Use.
2. Pathogenic microorganisms may be present in clinical specimens. All specimen and the related contaminated items need to be handled, stored and disposed following "Standard Precautions" and institutional guidelines.
3. This kit is unable to differentiate each of the *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti* from the others even if the *M. tuberculosis* complex test results are positive.
4. Do not use kit components beyond the expiration date.
5. Apply universal precaution when performing the test.
6. The test plate should be used immediately after opening the packaging. When it absorbs moisture, the quality deteriorates and an accurate result cannot be obtained.
7. Do not reuse the device.
8. Use a clean, sterile pipette tip for each sample.
9. Only AFB suspension (in extraction buffer) or AFB positive culture in liquid media can be applied as specimens. No clinical samples such as a human body fluid, tissue and bronchial lavage fluid can be directly used as specimens.
10. If the test is invalid, one should consider the possible improper handling, inaccurate operation procedure, or device quality. Repeat the test with a new device ensuring that the test procedure has been followed accurately.
11. Read the result at 15 minutes, or up to 60 minutes. Do not read the result longer than 60 minutes.

STORAGE CONDITIONS

Test devices must be stored at 2~30°C. **DO NOT FREEZE.** Devices must be at ambient room temperature at time of testing.

SAMPLE COLLECTION AND PREPARATION

Use a suspension of bacteria cultured on the medium for AFB or a bacterial culture liquid as a sample. No clinical samples such as a human body fluid, tissue or bronchial lavage fluid can be directly used, as they are as samples.

Sample Preparation from Positive Culture

1. Preparation of the sample from SOLID MEDIA

Colony

- (1) Select colonies that grow on solid media for 2~4 weeks.
- (2) Confirm the presence of AFB by acid-fast staining.
- (3) Dispense 0.2mL of TB MPT64 Extraction Buffer (Cat. No. M443105) into a tube.
- (4) Pick at least 1 μ L loop of colonies.
- (5) Suspend the colonies in the buffer solution in the tube.
- (6) Close the tube tightly and fully vortex the suspension.
- (7) Use the suspension as specimen.

If **EZER™ TB MPT64 Plus (Cat. No. P211010)** is used, adjust steps (3) and (4) as follows:

- (3) Take one extraction tube (containing 0.5 mL extraction buffer) and open the cap.
- (4) Using a 1 μ L loop, pick colonies three times and transfer them into the extraction tube.

Condensation fluid

If there is condensation fluid in the slant culture tube, 100 μ L of the condensation fluid can be directly applied to the sample well, or the colonies can be suspended in the condensation fluid, and then 100 μ L of the suspension can be applied.

1. Preparation of the sample from LIQUID MEDIA※

- (1) Confirm the presence of AFB by acid-fast staining.
- (2) Mix the culture positive tube by inverting.
- (3) Use the suspension as specimen.

※ Media listed below has been tested with no interference with the **EZER™ TB MPT64** test result.

- Egg-based media: Ogawa media, Lowenstein-Jensen (LJ) media.
- Agar media: Middlebrook 7H10 agar media, Middlebrook 7H11 agar media.
- Liquid media: Middlebrook 7H9 liquid media, Dubo's liquid media, Kirchner media, Sauton's media, BD BBL™ MGIT™ media.

PROCEDURE

1. Remove the **EZER™ TB MPT64** test device from its foil pouch immediately before use.
2. Lay the device on a flat surface.
3. Carefully apply 100 μ L specimen from AFB culture positive sample onto sample well.
4. Read results at 15 min or up to 60 min. Positive results may be reported as soon as 5 min while the test and control lines are visible.
5. Read in a well-lighted area and record the test result.

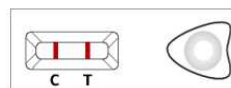
INTERPRETATION OF RESULTS

Allow the samples to react according to the procedure and read the red purple lines that appear in the reading area.

Positive

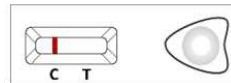
Two red lines appear. One red line appears in the control region (C), and one red line in the test region (T).

This indicates that the specimen contains detectable amount of MPT64 antigen. The shade of color may vary, but it should be considered positive whenever there is even a faint line.



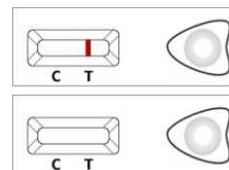
Negative

Only one reddish band appears on control region of the device. No reddish line is visible next to the Test "T". This indicates that there is no detectable MPT64 protein in the sample.



Invalid Result

No red line appears in the control region (C). The test is invalid even if there is a line in the region (T). Review testing procedures and repeat the test using a new rapid test device.



REPORTING OF RESULTS

Positive Test: Should be reported as MTb complex (MTbc).

Negative Test: Should be reported as Acid-Fast Bacilli, non-MTbc.

Invalid Test: Do not report results.

QUALITY CONTROL

Testing of positive and negative controls should be done immediately after receipt of the **EZER™ TB MPT64** antigen rapid test devices. This allows for checking the appropriate shipment conditions.

Internal control: Each EZER™ TB MPT64 antigen rapid test devices contains internal/procedural controls. The appearance of a control line at the Control “C” position validates the proper reagent function and assures that the correct test procedure was followed.

Positive control:

1. Prepare specimens from proven clinical isolate of *M. tuberculosis*.
2. Suspending 1µL (equivalent to the amount of a 1mm-diameter platinum micro-loop) of a colony of positive control isolate which grown on solid media in the sample extraction buffer.
3. A liquid culture of positive control isolate. (equivalent to a McFarland No.1 standard 3 to 6 x 10⁷CFU/mL).

Negative control:

1. Non-inoculated liquid media.
2. Sample extraction buffer.
3. Non-tuberculous *Mycobacteria* such as *M. smegmatis* or *M. goodii*.

※Only apply sample extraction buffer or noninoculated liquid media for negative control.

※Water and other reagent may have interference with the test result.

PERFORMANCE CHARACTERISTICS

Minimum detection limit

The minimum detection limit is 1.2 x 10⁶CFU/mL.

Cross-reactivity evaluation

The following Nontuberculous *Mycobacteria* and *Staphylococcus aureus* have been evaluated and revealed no cross-reactivity to the test:

<i>M. abscessus</i>	<i>M. asiaticum</i>	<i>M. avium</i>
<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. flavescens</i>
<i>M. goodii</i>	<i>M. intracellulare</i>	<i>M. kansasii</i>
<i>M. marinum</i> 329	<i>M. marinum</i> 351-2	<i>M. marinum</i> 60
<i>M. marinum</i> JATA22-01	<i>M. vaccae</i>	<i>M. xenopi</i>
<i>M. nonchromogenicum</i>	<i>M. terrae</i>	<i>M. ulcerans</i>
<i>M. scrofulaceum</i>	<i>M. simiae</i>	<i>M. smegmatis</i>
<i>M. szulgai</i>	<i>Sta. aureus</i>	

Reactivity with Standard Strains of *Mycobacterium tuberculosis* Complex Strains demonstrating the production of MPT64

A reaction was observed with the following 5 strains.

<i>M. tuberculosis</i> H37Rv	<i>M. tuberculosis</i> H37Ra
<i>M. africanum</i>	<i>M. microti</i>
<i>M. bovis</i>	

Clinical Studies

The EZER™ TB MPT64 test was compared to an immune-chromatographic assay method (Abbott

Bioline™ TB Ag MPT64) at one clinical site in China a total of 187 AFB smear-positive, MGIT 960 instrument positive samples.

Bioline™ TB Ag MPT64 identified 102 as MTbc and 85 as NTM. A comparison of these results is shown below in Table 1.

Table 1: Performance Summary of the EZER™ TB MPT64 test compared to Abbott Bioline™ TB Ag MPT64 with Percent Agreements

		Abbott TB Ag MPT64		
		+	–	Total
EZER™ TB MPT64	+	102	0	102
	–	0	85	85
	Total	102	85	187

Positive Percent Agreement: 100.00%

Negative Percent Agreement: 100.00%

Overall Percent Agreement: 100.00%

Interfering Substances

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, Lowenstein-Jensen (LJ) media
- Agar media : Middle Brook 7H10 agar media, Middle Brook 7H11 agar media
- Liquid media: Middle Brook 7H9 liquid media, Dubos liquid media, Kirchner media, Sauton's media

LIMITATIONS OF THE PROCEDURE

1. This test result not rule out the presence of other mycobacterial or mixed bacterial infections.
2. This test is unable to differentiate between *M. tuberculosis* complex (MTbc) organisms.
3. A negative result does not always rule out the possibility of infection with MTbc. The device is unable to detect MTbc when a mutation arises in the MPT64 gene. The test results are to be used in conjunction with information available from the patient's clinical evaluation and other diagnostic procedures.
4. Some sub-strains of *M. bovis* BCG among *M. tuberculosis* complex produce no MPT64 antigen and will therefore result in a negative test result with the device.
5. As with all diagnostic tests, the test results must always be correlated with clinical findings. The results of the test are to be interpreted within the epidemiological, clinical and therapeutic context.

6. Any modifications to the above procedure and/or use of other reagents might invalidate the test procedure.
7. Do not compare the intensity of the test line and control line to determine the concentration of the MPT64 protein in test specimen.

AVAILABILITY

Model	Cat. No.	Contents
EZER™ TB MPT64	P211009	40 Test Device
EZER™ TB MPT64 Plus	P211010	20 Test Device 20 Extraction Tube
TB MPT64 Extraction	M443105	10mL x 2

Each test device contains Colloidal gold-labeled anti-MPT64 monoclonal antibody and a control line of anti-species antibody.

REFERENCES

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8. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EECP). Office Journal L262, 17/10/2000, p. 0021-0045.
9. Hasegawa N, et al. New simple and rapid test for culture confirmation of Mycobacterium tuberculosis complex: a multicenter study. J Clin Microbiol. 2002; 40:908-912.

Index of Symbols

	Attention, see instructions for use		Tests per kit		Authorized Representative
	For in vitro diagnostic use only		Use by		Do not reuse
	Store between 2~30°C		Lot Number		Catalog #

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