AMPLIRUN® TOTAL MDR-TB VERIFICATION & CONTROL PANEL (SPUTUM)

For *in vitro* diagnostic use

MBTC027: Inactivated *Mycobacterium tuberculosis* (MTB) cells formulated to mimic human sputum specimen and intended to control sample processing, analysis and detection of *Mycobacterium tuberculosis* (TB) nucleic acids and genetic markers for drug resistant using the product as an external run control. MBTC027 panel is a 5-member panel; 1 sensitive, 2 rifampicin resistant and 2 isoniacid resistant strains.

INTRODUCTION:

Mycobacterium tuberculosis is a strictly aerobic, nonchromogenic, slowly-growing, acid fast bacillary bacterium.

Humans are the only reservoir of *M. tuberculosis*, an obligate pathogen that is transmitted by airborne particles and may remain latent for years before causing active tuberculosis.

CHARACTERISTICS:

The content is lyophilized. It is necessary to reconstitute it before use (refer to "Preparation of the reagents"). Total Controls are designed for single use, excess material should be discarded. Nucleic acid detection requires an extraction step that releases DNA/RNA for amplification and detection.

Product description:

MTB: Grown in Middlebrook 7H9 broth culture medium. Once purified, the cells are inactivated rendering them non-infectious and diluted in a human sputum matrix.

KIT CONTENTS:

1 VIRCELL TOTAL MTB CONTROL (SPUTUM): 2 vials with lyophilized cells of *M. tuberculosis* sensitive strain (20000-50000 copies/vial).

Lot number		
Concentration	copies/µl	

Table 1

2 VIRCELL TOTAL MTB RIF RESISTANT (531) CONTROL (SPUTUM): 2 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *rpoB* mutation (S531L) that confers rifampicin resistance.

Lot number		
Concentration	copies/µl	

Table 2

3 VIRCELL TOTAL MTB RIF RESISTANT (526) CONTROL (SPUTUM): 2 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *rpoB* mutation (H526D) that confers rifampicin resistance.

Lot number		
Concentration	copies/µl	

Table 3

4 VIRCELL TOTAL MTB INH RESISTANT (katG) CONTROL (SPUTUM): 2 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *katG* mutation (S315T) that confers isoniazid resistance.

Lot number		
Concentration	copies/µl	

Table 4

5 VIRCELL TOTAL MTB INH RESISTANT (inhA) CONTROL (SPUTUM): 2 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *inhA* mutation (C15T) that confers isoniazid resistance.

Lot number		
Concentration	copies/µl	

Table 5

M. tuberculosis quantification was performed by colony forming unit counting on Middlebrook agar plates.

Quantification validation was performed using a real-time PCR instrument.

Materials required but not supplied:

Molecular Biology grade water

Additional extraction and detection kit.

BD MAX™ instrument (optional)

STORAGE REQUIREMENTS:

Special transport conditions not required. Store the lyophilized vial at 2-8ºC. After reconstitution, suspension should be used on the same day. Unused product should be discarded.

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Use only the amount of reagent required for the test.

The product is stable until the expiry date indicated in the label, if the instructions for use are followed.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. This product is for *in vitro* diagnosis use only and for professional qualified staff.
2. Sterile tips with aerosol barrier are essential to prevent contamination.
3. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
4. In order to perform the test it is essential to have separate working areas.
5. Dispose of unused reagents and waste in accordance with all applicable regulations.
6. The component VIRCELL TOTAL CONTROL could include genetic material or substances of animal and/or human origin. VIRCELL TOTAL CONTROL contains inactivated microorganism, nevertheless, it should be considered potentially infectious and handled with care. Inactivation was verified by the absence of growth under same culture conditions used for each microorganism. No present method can offer complete assurance that these or other infectious agents are absent. All materials should be handled and disposed as of potentially infectious. Observe the local regulations for waste disposal.

PREPARATION OF THE REAGENTS:

1. Add 1000 µl of Molecular Biology grade water to vial 1, 2, 3, 4 or 5 and mix until completely reconstituted. The concentration will be approximately 35000 copies/ml once reconstituted.
2. Shake with vortex for 30 seconds to dissolve and homogenize completely.
3. Follow diagnostic kit instructions treating VIRCELL TOTAL CONTROL in an identical manner to a clinical specimen using recommended amount for extraction and detection.

Recommended protocol for BD MAX™ MDR-TB assay (optional)

The procedure for testing AmpliRun® TOTAL MDR-TB Verification & Control Panel in BD MAX™ instrument is the following:

1. Label a Sample Tube with the appropriate identification.
2. Using BSL2 procedure, rehydrate the Vircell control with 1ml of nuclease free water.
3. Carefully open BD MAX™ STR tube and add 2mL STR to the rehydrated control. The final ratio of STR to sample is 2:1.
4. Cap the vial and shake (do not vortex) the solution vigorously 10 times (up and down equals 1 time).
5. Incubate at room temperature for 5 minutes and shake vigorously again 10 times.
6. Incubate BD MAX™ STR-treated control at room temperature for 25 minutes.
7. Using the transfer pipet, transfer 2.5 mL of the STR-treated control to a labeled Sample Tube. Double check that the sample ID on the Sample Tube matches the label on control vial.
8. Close the Sample Tube with a blue septum cap.
9. Prepare any additional controls for testing by repeating Steps 1 through 10.
10. Proceed to BD MAX™ System Operation section to perform testing of the BD MAX™ MDR-TB on the BD MAX™ System.

EXPECTED RESULTS on BD MAX™ MDR-TB assay:

Panel Member	MTB Control Strain	BD MAX™ MDR-TB Assay Expected Result
1	*M. tuberculosis* sensitive strain	MTB detected RIF/INH Resistance NOT detected
2	*M. tuberculosis* rifampicin resistant strain (mutation in *rpoB* 531 residue)	MTB detected RIF Resistance Detected INH Resistance NOT Detected
3	*M. tuberculosis* rifampicin resistant strain (mutation in *rpoB* 526 residue)	MTB detected RIF Resistance Detected INH Resistance NOT Detected
4	*M. tuberculosis* isoniazid resistant strain (mutation in *katG*)	MTB detected RIF Resistance NOT Detected INH Resistance Detected
5	*M. tuberculosis* isoniazid resistant strain (mutation in *inhA* promoter)	MTB detected RIF Resistance NOT Detected INH Resistance Detected

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control testing before releasing. Quality control analysis is performed using a sample preparation kit and real-time PCR for quantification. Final quality control results for each particular lot are available.

INTERPRETATION OF RESULTS AND VALIDATION PROTOCOL FOR USERS:

Refer to indications of additional extraction and detection kit.

LIMITATIONS OF METHOD:

1. This reagent is intended to be used with methods of human diagnostics. This test has not been verified with other methods.
2. The user of this kit is advised to read carefully and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
3. Use of this product should be limited only to personnel trained in molecular techniques.
4. This external run control does not substitute internal diagnostic kit controls.
5. Quantification conclusions cannot be drawn from a single point sample of known concentration. Precise clinical sample quantification could only be achieved by the standard curve method using a calibrator as MBC034 AmpliRun® MYCOBACTERIUM TUBERCULOSIS DNA CONTROL.

PERFORMANCES:

• IDENTITY TEST

Performance analysis of TOTAL CONTROL:

Performance analysis was carried out by sample preparation and PCR analysis with a specific oligo pair for *M. tuberculosis*.

The reaction produced a 82 bp fragment. It was visualized on a 2% agarose gel using ethidium bromide staining.

• QUANTIFICATION TEST

Quantification is based on Real-Time qPCR using the standard curve method. This method involves the use of multiple replicates of different serial dilutions of both the product and the standard of quantification

• INTRA-ASSAY PRECISION

3 vials of the product were extracted under identical extraction conditions and 3 replicas of each extraction were amplified by the same operator under identical qPCR conditions. Less than 15% coefficient of variance was observed between all assays.

• INTER-ASSAY PRECISION

1 vial of the product was extracted and 3 replicates from this vial were amplified by 2 different operators on 3 consecutive days. Less than 15% coefficient of variance was observed between all assays.

BD MAX™ MDR-TB VALIDATION

Three unique batches of VIRCELL TOTAL MTB CONTROL were tested by two BD MAX™ operatos assigned to two BD MAX™ platforms, testing two different BD MAX™ MDR-TB assay reagents across both BD MAX™ platforms. All three batches of VIRCELL TOTAL MTB CONTROL were tested on each BD MAX™ run. There were 12 valid results per strain across three batches of controls.

The Percent Positive Agreement (PPA) was determined as follows;

100 x TP/TP + FN where TP = True Positive defined by VIRCELL TOTAL MTB CONTROL identification and FN = False Negative defined as BD MAX™ result not matching VIRCELL TOTAL MTB CONTROL identification.

Panel Member	BD MAX™ Batch 1 (x 2 users)			BD MAX™ Batch 2 (x 2 users)			Total N	PPA*
	1	2	3	1	2	3		
1	2	2	2	2	2	2	12	12/12=100%
2	2	2	2	2	2	2	12	12/12=100%
3	2	2	2	2	2	2	12	12/12=100%
4	2	2	2	2	2	2	12	12/12=100%
5	2	2	2	2	2	2	12	12/12=100%

*PPA: Percent Positive Agreement

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
RCNS Xµl	Reconstitute in x µl
SHIP	Shipment temperature
STORE	Storage temperature

BIBLIOGRAPHY:

1. Devonshire AS, O'Sullivan DM, Honeyborne I, Jones G, *et al.* (2016). The use of digital PCR to improve the application of quantitative molecular diagnostic methods for tuberculosis. BMC Infect Dis. 2016 Aug 3;16:366.

2. Zimmermann S, Dalpke AH, Murray P,Cooper C (2018). Pre-validation of the BD MAX MDR-TB assay for the rapid detection of MTBc DNA and mutations associated with rifampin and isoniazid resistance. Poster presented at 29th ECCMID (Madrid).

3. Scott L, Albert H, Gilpin C, Alexander H, DeGruy K, Stevens W. (2014). Multicenter feasibility study to assess external quality assessment panels for Xpert MTB/RIF assay in South Africa. J Clin Microbiol. 2014 Jul;52(7):2493-9.

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