

AMPLIRUN® TOTAL MTB RIF RESISTANT CONTROL (SPUTUM)

For *in vitro* diagnostic use

MBTC014: Rifampicin resistant inactivated *Mycobacterium tuberculosis* (MTB) cells formulated to mimic human sputum specimen and intended to validate and control sample processing, analysis and detection of nucleic acid assays based on the identification gene mutations associated with rifampicin resistant MTB, using the product as an external run control.

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 an 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 RIF RESISTANT (531) CONTROL (SPUTUM): 5 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *rpoB* mutation (S531L) that confers rifampicin resistance. Batch concentration is provided in Certificate of Analysis.

2 VIRCELL TOTAL MTB RIF RESISTANT (526) CONTROL (SPUTUM): 5 vials with lyophilized cells of *M. tuberculosis* (20000-50000 copies/vial) harboring a *rpoB* mutation (H526D) that confers rifampicin resistance. Batch concentration is provided in Certificate of Analysis.

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

Quantification validation was performed by real-time PCR.

Materials required but not supplied:

Molecular Biology grade water
Additional extraction and detection kit.

STORAGE REQUIREMENTS:

Special transport conditions not required. Store the lyophilized vial at 2-8°C. 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 each vial 1 or 2 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 TOTAL CONTROL in an identical manner to a clinical specimen using recommended amount for extraction and detection.

GeneXpert® MTB/RIF:

1. Add 1000 µl of Molecular Biology grade water to each vial 1 or 2 and mix until completely reconstituted. The concentration will be approximately 35000 copies/ml once reconstituted.
2. Add 2000 µl of SR buffer to the resuspended control.
3. Mix thoroughly by inverting 10-20 times.
4. Incubate for 5 minutes at room temperature.
5. Mix again by inverting 10-20 times.
6. Incubate for a further 10 minutes at room temperature.
7. Add 2000 µl of the reconstituted control solution to the GeneXpert® MTB/RIF cartridge.

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.
6. AMPLIRUN® TOTAL has not been designed to be used with a particular diagnostic kit coming from a certain manufacturer. It is used to validate and control sample processing, analysis and detection of a diagnostic laboratory functioning procedure.

PERFORMANCES:

• IDENTITY TEST

PCR analysis was performed after extraction with a specific oligonucleotide pair for *M. tuberculosis*. The reaction produced a fragment of the expected size.

• 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







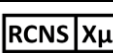


• INTRA-ASSAY PRECISION

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.

SYMBOLS USED IN LABELS:

	<i>In vitro</i> diagnostic medical device
	Use by (expiration date)
	Store at x-y°C
	Batch code
	Catalogue number
	Consult instructions for use
	Reconstitute in x µl
	Shipment temperature
	Storage temperature

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5. Sanjuan-Jimenez R, Colmenero JD, Bermúdez P, Alonso A, Morata P. (2013). Amplicon DNA melting analysis for the simultaneous detection of *Brucella* spp and *Mycobacterium tuberculosis* complex. Potential use in rapid differential diagnosis between extrapulmonary tuberculosis and focal complications of brucellosis. *PLOS ONE* March 2013. Volume 8 Issue 3.

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