

COBAS® TaqMan® MTB Test



FOR *IN VITRO* DIAGNOSTIC USE.

COBAS® TaqMan® MTB Test	CTM MTB	48 Tests	P/N: 04803531 190
AMPLICOR® Respiratory Specimen Preparation Kit	RSP PREP	100 Tests	P/N: 20756903 122 ART: 07 5690 3 US: 83267

INTENDED USE

The COBAS® TaqMan® MTB Test is an *in vitro* nucleic acid amplification test for the qualitative detection of *Mycobacteria tuberculosis* (MTB) complex DNA in liquefied, decontaminated and concentrated human respiratory specimens, including sputum and bronchial alveolar lavages (BAL). The test utilizes the AMPLICOR® Respiratory Specimen Preparation Kit for manual specimen preparation and the COBAS® TaqMan® 48 Analyzer for automated amplification and detection.

SUMMARY AND EXPLANATION OF THE TEST

Members of the genus *Mycobacterium* are acid-fast, non-motile, rod-shaped, aerobic bacteria that do not form spores. They contain several species pathogenic to humans. Many mycobacterial species are soil and water saprophytes. The primary human pathogens are those species of the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*), and *M. leprae*. *M. tuberculosis* is the most important mycobacterial pathogen and is only found in humans.

Tuberculosis (TB) is a contagious disease. Like the common cold, it spreads through the air. Only people who are sick with TB in their lungs are infectious. When infectious people cough, sneeze, talk or spit, they propel TB germs into the air. A person needs only to inhale a small number of these to be infected. Overall, one-third of the world's population is currently infected with the TB bacillus. Of the people who are infected with TB bacilli, 5-10% become sick or infectious at some time during their life. It is estimated that 1.7 million deaths resulted from TB in 2004. As with cases of disease, the highest number of estimated deaths is in the South-East Asia Region, but the highest mortality per capita is in the Africa Region, where HIV has led to rapid increases in the incidence of TB and increases in the likelihood of dying from TB. WHO targets, ratified by the World Health Assembly in 1991, are to detect 70% of new infectious TB cases and to cure 85% of those detected by 2005. Eighteen countries had already achieved these targets in 2002¹⁻⁵.

Due to the risk of the spread of the disease, the potential for the emergence of drug-resistant strains, and the severity of the disease in patients infected with HIV-1, the rapid diagnosis of the *M. tuberculosis* complex is extremely important.

The isolation of an organism from the *M. tuberculosis* complex is required for the definitive diagnosis of tuberculosis. Routine cultures are time-consuming and can take up to eight weeks. Microscopic examination of acid-fast smears is the most rapid method for the detection of mycobacteria, but it is insensitive and non-specific. Immunological and serological techniques are limited, in general, due to poor sensitivity and/or specificity^{6,7}. The development of PCR-based tests specific for mycobacteria has been shown to further improve the rapid diagnosis of tuberculosis by allowing the direct detection of mycobacteria in clinical specimens⁸⁻¹⁴.

PRINCIPLES OF THE PROCEDURE

The COBAS® TaqMan® MTB Test is based on two major processes: (1) manual specimen preparation to obtain MTB DNA; (2) simultaneous PCR amplification of target DNA using complementary primers, and detection of target DNA through cleavage of dual fluorescent dye-labeled oligonucleotide probes^{15,16}. Together, these processes permit the detection of MTB target amplified product (amplicon) and *Mycobacterium* Internal Control DNA, which is amplified and detected simultaneously with the specimen.

The Master Mix reagent contains a primer pair that is used to amplify a genus-specific region of the chromosome and specificity for the *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*) is provided by a dual labeled target detection probe. The same primer pair is used to amplify the Internal Control DNA. The detection of amplified DNA is performed using target-specific and Internal Control-specific dual labeled oligonucleotide probes that permit independent identification of MTB amplicon and *Mycobacterium* Internal Control amplicon.

Specimen Preparation

NALC/NaOH¹⁷ liquefied, decontaminated sputum and BAL specimens are washed with Respiratory Specimen Wash Solution. Organisms are lysed by incubation in Respiratory Specimen Lysis Reagent and the specimen is made amplification ready by the addition of Respiratory Specimen Neutralization Reagent.

The Document Revision Information section is located at the end of this document.

PCR Amplification

Target Selection

The *Mycobacterium* genome contains a highly conserved region of approximately 1500 nucleotides encoding the gene for 16S rRNA. The COBAS® TaqMan® MTB Test uses *Mycobacterium* genus specific primers to define a sequence within this region¹⁸.

Target Amplification

PCR amplification occurs in K-tubes or K-trays in which prepared specimens are added to the amplification mixture. The reaction mixture is heated to denature the double stranded DNA and expose the primer target sequences. As the mixture cools, the primers anneal to their target sequences. Z05 DNA polymerase, in the presence of Mg²⁺ and excess deoxynucleotide triphosphates (dNTPs), extends the annealed primers along the target templates to produce a double stranded DNA molecule termed an amplicon. The COBAS® TaqMan® 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of cycles is preprogrammed into the COBAS® TaqMan® 48 Analyzer. Amplification occurs only in the region of the MTB genome between the primers; the entire MTB genome is not amplified.

Internal Control Amplification

In enzyme-based amplification processes such as PCR, efficiency can be reduced by inhibitors that may be present in the clinical specimen. The Internal Control permits the identification of prepared specimens containing substances that may interfere with PCR amplification. The *Mycobacterium* Internal Control is a non-infectious, recombinant linearized plasmid DNA with primer binding regions identical to those of the *M. tuberculosis* target sequence, a randomized internal sequence of similar length and base composition as the *M. tuberculosis* target sequence, and a unique probe binding region that differentiates the *Mycobacterium* Internal Control amplicon from target amplicon. These features are selected to ensure equivalent amplification of both *Mycobacterium* Internal Control and the *M. tuberculosis* target DNA. The *Mycobacterium* Internal Control Reagent is included in the COBAS® TaqMan® MTB Test and is introduced into each amplification reaction to be co-amplified with MTB DNA from the clinical specimen. The *Mycobacterium* Internal Control is designed to ensure identification of specimens that contain inhibitors that would interfere with the amplification and detection of the MTB target sequence.

Selective Amplification

Selective amplification of target nucleic acid from the specimen is achieved in the COBAS® TaqMan® MTB Test by use of AmpErase (uracil-N-glycosylase) enzyme¹⁹ and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing thymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contains deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by the AmpErase enzyme prior to amplification of the target DNA. Any nonspecific product formed after initial activation of the Master Mix by the magnesium is destroyed by the AmpErase enzyme. The AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at deoxyuridine residues by opening the deoxyribose chain at the C1- position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme is inactive at temperatures above 55°C, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon formed during amplification.

Detection of PCR Products in a COBAS® TaqMan® Test

The COBAS® TaqMan® MTB Test utilizes real-time PCR technology. The use of dual-labeled fluorescent probes provides for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of MTB and *Mycobacterium* Internal Control-specific oligonucleotides labeled with a reporter dye and a quencher dye. In the COBAS® TaqMan® MTB Test, the MTB and *Mycobacterium* Internal Control probes are labeled with different fluorescent reporter dyes. When the dual fluorescent dye-labeled probes are intact, the reporter fluorescence is suppressed by the proximity of the quencher dye due to Förster-type energy transfer effects. During PCR, the probe hybridizes to a target sequence and is cleaved by the 5' to 3' exonuclease activity of the thermostable Z05 DNA polymerase. Once the reporter and quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of MTB DNA and *Mycobacterium* Internal Control DNA are measured independently at different wavelengths. This process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of MTB DNA and Internal Control DNA.

REAGENTS**AMPLICOR® Respiratory Specimen Preparation Kit**
(P/N: 20756903 122; ART: 07 5690 3; US: 83267)

RSP PREP

100 Tests**RW**

(Respiratory Specimen Wash Solution)

Tris-HCl buffer
< 1% Solubilizer
EDTA
0.05% Sodium azide

2 x 25 mL

RL

(Respiratory Specimen Lysis Reagent)

0.2% Sodium hydroxide
< 1% Solubilizer
EDTA
0.05% Sodium azide

3 x 6 mL

RN

(Respiratory Specimen Neutralization Reagent)

Tris-HCl buffer
Magnesium chloride
0.05% Sodium azide

2 x 5 mL

COBAS® TaqMan® MTB Test

(P/N: 04803531 190)

CTM MTB

48 Tests**Control Reagents****MYCO IC**

(Mycobacterium Internal Control)

Tris-HCl buffer
< 0.001% Non-infectious plasmid DNA (microbial) containing
Mycobacterium primer binding sequences and a unique probe
binding region
< 0.005% Poly rA RNA (synthetic)
EDTA
Amaranth dye
0.05% Sodium azide

4 x 0.1 mL

MTB (+) C[*M. tuberculosis* Positive Control]Tris-HCl buffer
< 0.001% Non-infectious plasmid DNA (microbial) containing
MTB sequences
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

1 x 0.75 mL

MYCO (-) C

[Mycobacterium Negative Control]

Tris-HCl buffer
< 0.005% Poly rA RNA (synthetic)
EDTA
0.05% Sodium azide

1 x 0.75 mL

Amplification and Detection Reagents

MTB MMX

(COBAS® TaqMan® MTB Master Mix)

4 x 12 Tests

Tricine buffer

4 x 0.46 mL

Potassium acetate

Potassium hydroxide

Dimethyl sulfoxide

Glycerol

< 0.001% dATP, dCTP, dGTP, dUTP

< 0.001% MTB primers

< 0.001% Fluorescent-labeled oligonucleotide probes specific for MTB and the Mycobacterium Internal Control

< 0.001% Oligonucleotide aptamer

< 0.05% Z05 DNA Polymerase (microbial)

< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)

0.09% Sodium azide

MYCO Mg²⁺

(Mycobacterium Magnesium Reagent)

4 x 12 Tests

<1.0% Magnesium acetate

4 x 0.2 mL

0.09% Sodium azide

WARNINGS AND PRECAUTIONS

A. FOR *IN VITRO* DIAGNOSTIC USE.

- B. This test is for use only with sputum or BAL that has been liquefied, decontaminated and concentrated using NALC-NaOH¹⁷ methods.
- C. Do not pipette by mouth.
- D. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
- E. Avoid microbial contamination of reagents when removing aliquots from reagent bottles.
- F. The use of sterile disposable pipettes and pipette tips is recommended.
- G. Do not pool reagents from different lots or from different bottles of the same lot.
- H. Do not mix reagents from different kits.
- I. Dispose of unused reagents, waste and specimens in accordance with country, federal, state and local regulations.
- J. Do not use a kit after its expiration date.
- K. Material Safety Data Sheets (MSDS) are available on request from your local Roche office.
- L. Specimens and controls should be handled as if infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*²⁰ and in the CLSI Document M29-A3²¹. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.

NOTE: Commercial liquid household bleach typically contains sodium hypochlorite at a concentration of 5.25%. A 1:10 dilution of household bleach will produce a 0.5% sodium hypochlorite solution.

- M. **RW, RL, RN, MTB MMX, MYCO Mg²⁺, MYCO IC, MTB (+) C and MYCO (-) C** contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of sodium azide containing solutions down laboratory sinks, flush the drains with a large volume of water to prevent azide buildup.
- N. Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens. **Do not use snap cap tubes.**
- O. Wear eye protection, laboratory coats and disposable gloves when handling any reagent. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills of these reagents occur, dilute with water before wiping dry.
- P. If a K-tube or K-tray ever opens after amplification treat the K-carrier, wells and the released liquid with bleach (effective concentration is 5000 ppm; 0.5%) overnight, to destroy the released amplicon.

STORAGE AND HANDLING REQUIREMENTS

Do not freeze reagents or controls.

- A. Store **RW, RL** and **RN** at 2-25°C. Unopened, these reagents are stable until the expiration date indicated.
- B. Store **MTB MMX, MYCO Mg²⁺, MYCO IC, MTB (+) C** and **MYCO (-) C** at 2-8°C. Unopened, these reagents are stable until the expiration date indicated.
- C. Store partially used **MTB (+) C** and **MYCO (-) C** at 2-8°C between runs. Check expiration date of opened controls prior to use. **MTB (+) C** and **MYCO (-) C** may be used up to four times. Once opened, use **MTB (+) C** and **MYCO (-) C** within 4 weeks or until the expiration date, whichever comes first.
- D. **MTB MMX, MYCO Mg²⁺** and **MYCO IC** are supplied as single use vials for sets of 12 reactions. Any unused material must be discarded.
- E. Working Master Mix (prepared by the addition of **MYCO Mg²⁺** and **MYCO IC** to **MTB MMX**) can be stored in the dark at 2-8°C for up to 2 hours.
- F. Prepared specimens and controls must be added to working Master Mix within 2 hours of preparation if stored at room temperature or within 8 hours if stored at 2-8°C.
- G. Amplification must be started within 90 minutes from the time that the processed specimens and controls are added to the Working Master Mix.

MATERIALS PROVIDED

Specimen Preparation Reagents

- A. **AMPLICOR® Respiratory Specimen Preparation Kit**
(P/N: 20756903 122; ART: 07 5690 3; US: 83267)

RSP PREP

RW

(Respiratory Specimen Wash Solution)

RL

(Respiratory Specimen Lysis Reagent)

RN

(Respiratory Specimen Neutralization Reagent)

Amplification and Detection Reagents

- B. **COBAS® TaqMan® MTB Test**
(P/N: 04803531 190)

CTM MTB

MYCO IC

(Mycobacterium Internal Control)

MTB (+) C

[*M. tuberculosis* Positive Control]

MYCO (-) C

[Mycobacterium Negative Control]

MTB MMX

(COBAS® TaqMan® MTB Master Mix)

MYCO Mg²⁺

(Mycobacterium Magnesium Reagent)

MATERIALS REQUIRED BUT NOT PROVIDED

Instrumentation and Software

- COBAS® TaqMan® 48 Analyzer
- AMPLILINK software Version 3.2 or 3.3 Series
- Data station for the AMPLILINK software
- COBAS® TaqMan® 48 analyzer Instrument Manual For use with the AMPLILINK software, version 3.2 and 3.3 series Application Manual
- AMPLILINK Software Version 3.2 Series Application Manual For use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, and COBAS® AMPLICOR® Analyzer

or

- AMPLILINK software Version 3.3 Series Application Manual For use with COBAS® AmpliPrep instrument, COBAS® TaqMan® analyzer, COBAS® TaqMan® 48 analyzer, COBAS® AMPLICOR® analyzer, and **cobas p** 630 instrument
- K-tube capper (P/N: 03516539001)
- K-tray capping tool (P/N: 03339904001)
- COBAS® TaqMan® 48 Analyzer K-carrier (P/N: 28150397001)
- COBAS® TaqMan® 48 Analyzer K-tray carrier (P/N: 03341488001)

Disposables

- K-tubes (P/N: 03137082001)
- COBAS® TaqMan® 48 Analyzer K-trays (P/N: 03343146001)

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

- Vortex mixer
- Pipette Aid: Drummond (P/N: 4-000-100) or equivalent
- 1.5 mL polypropylene screw-cap tubes, sterile, non-siliconized, conical (Sarstedt 72.692.105 or equivalent)**
- Tube racks (Sarstedt 93.1428 or equivalent)
- Pipettors (capacity 50 µL, 100 µL, 200 µL, 250 µL, 500 µL and 1000 µL)* with aerosol barrier or positive displacement tips
- Sterile, fine-tip transfer pipets
- Microcentrifuge (max. RCF 16,000 x g, min. RCF 12,500 x g); Eppendorf 5415C, HERMLE Z230M, or equivalent
- 60°C ± 2°C heating block
- Thermometer
- Absorbent paper
- Disposable gloves, powderless
- Biosafety cabinet with negative pressure

* Pipettors should be accurate within 3% of stated volume. Aerosol barrier or positive displacement DNase-free (or nuclease-free) tips must be used where specified to prevent specimen and amplicon cross-contamination.

** Screw-cap tubes must be used for specimen and control preparation to prevent splashing and potential cross-contamination of specimens and controls. **Do not use snap cap tubes.**

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

NOTE: Handle all specimens and controls as if they are capable of transmitting infectious agents.

A. Specimen Collection

The only acceptable respiratory specimen is expectorated and induced sputum or BAL. This specimen must be liquefied, decontaminated and concentrated using NALC-NaOH¹⁷ methods.

B. Specimen Transport

Decontaminated specimens must be transported to the laboratory at 2-25°C within 24 hours of collection. If the specimens are not transported within 24 hours, they must be stored and shipped at -70°C. Specimens must be shipped in compliance with all country, federal, state and local regulations for the transport of etiologic agents²².

C. Specimen Storage

Decontaminated specimens can be stored at -20°C for up to 2 weeks.

INSTRUCTIONS FOR USE

NOTE: For detailed operating instructions, a detailed description of the possible configurations, printing results and interpreting flags, comments and error messages, refer to (1) the COBAS® TaqMan® 48 analyzer Instrument Manual For use with the AMPLILINK software, version 3.2 and 3.3 series Application Manual; (2) the AMPLILINK Software Version 3.2 Series Application Manual For use with the COBAS® AmpliPrep Instrument, COBAS® TaqMan® Analyzer, COBAS® TaqMan® 48 Analyzer, and COBAS® AMPLICOR® Analyzer or the AMPLILINK software Version 3.3 Series Application Manual For use with COBAS® AmpliPrep instrument, COBAS® TaqMan® analyzer, COBAS® TaqMan® 48 analyzer, COBAS® AMPLICOR® analyzer, and cobas p 630 instrument.

NOTE: If specimen preparation, amplification and detection are performed in a single work day, follow the test procedure as described below. If specimen preparation is performed on a separate day from amplification and detection, perform Reagent Preparation on the same day as Amplification and Detection.

NOTE: All amplification and detection reagents must be at ambient temperature before use; remove from 2 - 8°C storage at least 30 minutes before use.

NOTE: Specimens must be thawed (if frozen), equilibrated for 15 - 30 minutes at ambient temperature and mixed before use.

NOTE: Use pipettors with aerosol barrier or positive displacement tips where specified. Use extreme care to avoid contamination.

Run Size:

Each COBAS® TaqMan® MTB Test kit contains reagents sufficient for four 12-test runs, which may be performed separately or simultaneously. One replicate each of the **MYCO (-) C** and the **MTB (+) C** must be included in each test run of up to 48 specimens and controls (see "Quality Control" section). The Amplification and Detection Reagents are packaged in 12-test, single use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12.

A. Specimen and Control Preparation

Performed in: Pre-Amplification - Specimen and Control Preparation Area

1. Determine the number of specimens to be tested and arrange sufficient 1.5 mL polypropylene screw-cap tubes in the work area to have one tube for each specimen and one tube for each control. **Do not use snap cap tubes.** See "Warnings and Precautions." Label each specimen preparation tube with the specimen identification number.
2. Add 500 µL **RW** to each specimen tube.
3. Using a pipettor with an aerosol barrier pipet tip, add 100 µL liquefied, decontaminated and concentrated respiratory specimen to the appropriately labeled tube containing **RW**. Use a new aerosol barrier pipet tip for each specimen. Recap the tubes and vortex for 5 seconds.
4. Centrifuge at 12,500 x g for 10 minutes.
5. Aspirate supernatant using a fine-tip transfer pipet and add 100 µL **RL** to the cell pellet using a pipettor with a new aerosol barrier pipet tip for each specimen. Vortex for 5 seconds to resuspend the pellet.
6. Prepare the Working Controls as follows:

NOTE: Working Controls must be prepared fresh each day the test is performed and must be discarded at the end of the day.

NOTE: The **MTB (+) C** and **MYCO (-) C** provided in the COBAS® TaqMan® MTB Test kit are designed to be used up to four times to prepare **MTB (+)** and **MYCO (-)** Working Controls. Once opened, use **MTB (+) C** and **MYCO (-) C** within 4 weeks or until expiration date, whichever comes first.

- a. Vortex **MYCO (-) C** vial for 5 seconds. Pipet 50 µL **MYCO (-) C** into a 1.5 mL polypropylene tube using a pipettor with an aerosol barrier tip. Add 200 µL **RL**. Vortex for 5 seconds. This is the *Mycobacterium* (-) Working Control.
- b. Vortex the **MTB (+) C** vial for 5 seconds. Pipet 50 µL **MTB (+) C** into a 1.5 mL polypropylene tube using a pipettor with an aerosol barrier tip. Add 200 µL **RL**. Vortex for 5 seconds. This is the *M. tuberculosis* (+) Working Control.

- c. Pipet 100 µL of each Working Control into a 1.5 mL polypropylene screw-cap tube to be prepared in parallel with the clinical specimens.
7. Incubate specimens and controls in a 60°C ± 2°C dry heat block for 45 minutes.
8. Remove tubes from heat block and pulse centrifuge the tubes for 5 seconds to remove condensate from the cap.
9. Using a pipettor with an aerosol barrier pipet tip, add 100 µL **RN** to each specimen and control tube. Vortex for 5 seconds at half speed. Use a new aerosol barrier pipet tip for each specimen and control.
10. Move the prepared samples (patient specimens and controls) to the Amplification/Detection Area.

B. Reagent Preparation and Loading of K-carrier

Performed in: Pre-Amplification - Reagent Preparation Area

NOTE: COBAS® TaqMan® MTB Master Mix (MTB MMX), “Working Master Mix” (Working MMX) and Working MMX plus prepared specimens and controls are light sensitive. Protect these reagents from light.

NOTE: COBAS® TaqMan® MTB Master Mix (MTB MMX), COBAS® TaqMan® Mycobacterium Internal Control (MYCO IC) and COBAS® TaqMan® Mycobacterium Magnesium Reagent (MYCO Mg²⁺) must be equilibrated at ambient room temperature for at least 30 minutes prior to preparation of Working Master Mix.

NOTE: Prepare Working MMX after completion of manual Specimen and Control Preparation.

NOTE: Prepared specimens are stable for up to 4 hours at room temperature or up to 4 days at 2-8°C before addition into Working Master Mix.

NOTE: The Working MMX must be stored at 2-8°C and used within 2 hours of preparation.

NOTE: Prepared specimens can be stored at -70°C for up to 6 months.

NOTE: Once prepared specimens and controls are added to Working MMX, amplification must be started within 90 minutes.

1. Equilibrate one vial of **MTB MMX**, one vial of **MYCO Mg²⁺** and one vial of **MYCO IC** at ambient temperature for at least 30 minutes.
2. Place K-carrier in a K-carrier holder.
3. Place new K-tubes or a new K-tray in the K-carrier without touching the sides of the K-tubes or K-tray wells.

NOTE: If fewer than 24 assays are to be run, K-tube well positions 1, 2, 5, 20, 23 and 24 must be occupied to balance the K-carrier in the Thermal Cycler.

4. If applicable, uncap the K-tubes using the K-tube Capper. Place caps in the K-tube Park Position Holder.
5. Prepare the Working MMX as follows:
Add 200 µL of **MYCO Mg²⁺** and 50 µL **MYCO IC** into one vial **MTB MMX**. Cap the tube and mix well by inverting 10 times. Do not vortex the Working MMX. Protect the Working Master Mix from light and use within 2 hours.
6. Pipette 50 µL of Working MMX into each K-tube or appropriate K-tray well.

NOTE: Working MMX is very light sensitive. Minimize light exposure of K-tubes or K-tray containing Working MMX.

7. Add 50 µL of each prepared specimen or control to the appropriate K-tube or K-tray well containing Working MMX using a micropipettor with an aerosol barrier or positive displacement tip. Gently mix each specimen or control up and down three times with the micropipettor without generating bubbles.
8. Repeat step 7 for each prepared specimen and prepared control until all have been transferred to K-tubes or K-trays. Use a new tip for each specimen and control. Visually inspect for bubbles and remove as necessary. Cap the K-tubes/K-trays using a K-tube Capper/K-tray capping tool. Visually verify appropriate volumes have been added.
9. Store the PCR ready K-carrier in the dark at 15-30 °C if amplification does not start immediately. Amplification must be started within 90 minutes after prepared specimens and controls are added to the K-tubes or K-tray containing the Working MMX.

C. Amplification and Detection

Performed in: Post-Amplification - Amplification/Detection Area

COBAS® TaqMan® 48 Analyzer Loading and Operation

1. Turn on the workstation computer and log onto Windows XP using the appropriate User ID and password.
2. Turn on COBAS® TaqMan® 48 Analyzer at least 30 minutes prior to starting the run. Verify that the instrument initializes and is ready for use. If K-carriers from previous run(s) are still located in either of the Thermal Cyclers, remove them using the K-carrier transporter.
3. Open AMPLILINK software on the computer. Log on using the appropriate User ID and password.
4. To create K-carrier orders for the specimens to be analyzed, click on the **Orders** icon. Select the **Sample** tab, and then click on the **New** button and enter the order number for each specimen using the keypad or barcode scanner. Select the Test Definition for the COBAS® TaqMan® MTB Test. Repeat for each specimen. Click the **Save** button.

NOTE: If fewer than 24 assays are to be run, K-tube well positions 1, 2, 5, 20, 23 and 24 must be occupied to balance the K-carrier in the Thermal Cycler.

5. Enter Quality Control information by selecting the **Quality Control** tab in the Orders window. Click the **New** button and enter the information from the COBAS® TaqMan® MTB Test Value Card supplied with the kit using the keypad or barcode scanner. Enter the COBAS® TaqMan® MTB Test lot number, expiration date, Low (+) Control range as well as calibration coefficients in the designated spaces. Click **OK**.
6. Assign a K-carrier number for the run by clicking on the **K-Carrier** tab in the Orders window. In the **K-Carrier** window, click **New**. In the cell to the right of **"K-Carrier ID"**, enter the K-carrier number from the barcode on the K-carrier or the K-tray using the keypad or barcode scanner. Select the Test Definition for the COBAS® TaqMan® MTB Test from the test panel at the lower portion of the window.
7. In the **Worklist** select the first row of the Type (T) column. Highlight this field to access the pull down menu and then select the required control type. Next, double click the sample ID field for the same row; the **LookUp Control** window will be displayed with all available controls. When the control is selected, the corresponding calibration and control values will be displayed in the lower right Information panel. Repeat this process for all required controls.
8. To enter specimens to the **Worklist** double-click on the first position (row) for specimen entry. This will display the **Lookup Sample** window containing the assigned specimen orders; use the **Shift + Arrow** keys to highlight more than one order number. Verify that all orders have been assigned the Test Definition for the COBAS® TaqMan® MTB Test.
9. Click **Save** to save the K-carrier order assignment.
10. Select the **Systems** Icon in the System Tab; click **Open** to open the Thermal Cycler. When the Thermal Cycler Cover has completely opened and **"Ready to Load"** is seen in the **Systems** window, lift and hold the Thermal Cycler lid open. Using the K-carrier Transporter, transfer the loaded K-carrier containing the capped K-tubes with Working Master Mix and specimens and controls into the Thermal Cycler. Close the Thermal Cycler lid.
11. Click **Start** on the **Systems** window below the TC icon to close the Thermal Cycler Cover and start the run.
12. Amplification and detection are automatically performed by the COBAS® TaqMan® 48 Analyzer.

RESULTS

Result Calculation Using AMPLILINK version 3.2 series

The COBAS® TaqMan® 48 Analyzer automatically determines whether MTB DNA is detected for the specimen or control. With the introduction of AMPLILINK 3.3 series, a new qualitative result feature is used for the COBAS® TaqMan® MTB Test. The results presentation is different between the AMPLILINK version 3.2 series and AMPLILINK version 3.3 series.

The COBAS® TaqMan® 48 Analyzer:

- Determines the Cycle Threshold value (Ct) for the MTB DNA and the Mycobacterium IC DNA.
- Determines whether MTB DNA is detected based upon the Ct values for the MTB DNA and MYCO IC DNA.
- Determines if **MTB (+) C** and **MYCO (-) C** are valid.

Run Validation for AMPLILINK version 3.2 series

Check AMPLILINK results window or printout for flags and comments to ensure that the run is valid.

The run is valid if no flags appear for the COBAS® TaqMan® MTB Controls. The following results are obtained for a valid run:

Control	Result	Interpretation
Negative Control	Target Not Detected	Control within Range
Positive Control	1 Positive	Control within Range

The run is not valid if any of the following flags appear for the COBAS® TaqMan® MTB Controls:

MYCO Negative Control:

Flag	Result	Interpretation
_N_NC_INVALID	Invalid	Contamination or no IC signal

MTB Positive Control:

Flag	Result	Interpretation
_L_LPC_INVALID	Invalid	Control not within Range, no target signal or no IC signal NOTE: MTB PC is referred to as low positive control (LPC)

If the run is invalid, repeat the entire run including specimen and control preparation, amplification and detection.

Interpretation of Results:

For a valid run, check each individual specimen for flags or comments on the result printout. Interpret the results as follows:

- A valid run may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Flag	Result	Interpretation
No flag	Target Not Detected	<i>M. tuberculosis DNA not detected.</i> Specimen is presumptive negative for <i>M. tuberculosis</i> . A negative result does not preclude <i>M. tuberculosis</i> infection because results depend on adequate specimen collection, absence of inhibitors and sufficient DNA to be detected.
No flag	1 Positive	<i>M. tuberculosis DNA detected.</i> Specimen is positive for <i>M. tuberculosis</i> .
_Q_RFITOOLOW or No flag	> 1 Positive	<i>M. tuberculosis DNA detected.</i> Specimen is positive for <i>M. tuberculosis</i> .
_Q_QS_INVALID	Invalid	<i>Inhibitory Specimen.</i> <i>M. tuberculosis</i> DNA, if present, would not be detectable. Process another aliquot of the original specimen and repeat the test. Inhibitors are often labile and specimens initially inhibitory may not be inhibited when repeated. If the original specimen is not available, a new specimen must be collected. Note: AMPLILINK software flags IC failure as QS invalid.

Result Calculation Using AMPLILINK version 3.3 series

The COBAS® TaqMan® 48 Analyzer automatically determines whether MTB DNA is detected for the specimen or control. With the introduction of AMPLILINK version 3.3 series, a new qualitative result feature is used for the COBAS® TaqMan® MTB Test. The results presentation is different between AMPLILINK version 3.3 series and AMPLILINK version 3.2 series.

The COBAS® TaqMan® 48 Analyzer:

- Determines the Cycle Threshold value (Ct) for the MTB DNA and the Mycobacterium IC DNA.
- Determines whether MTB DNA is detected based upon the Ct values for the MTB DNA and MYCO IC DNA.
- Determines if **MTB (+) C** and **MYCO (-) C** are valid.

Run Validation for AMPLILINK version 3.3 series

Check AMPLILINK results window or printout for flags and comments to ensure that the run is valid.

The run is valid if no flags appear for the COBAS® TaqMan® MTB Controls. The following results are obtained for a valid run:

Control	Result	Interpretation
Negative Control	Negative	Control within Range
Positive Control	Positive	Control within Range

The run is not valid if any of the following flags appear for the COBAS® TaqMan® MTB Controls:

MYCO Negative Control:

Flag	Result	Interpretation
NC_INVALID	Invalid	No IC signal or IC signal out of range

MTB Positive Control:

Flag	Result	Interpretation
PC_INVALID	Invalid	Control not within Range, no target signal or no IC signal

If any control is invalid, all specimens in the run will be reported as INVALID. Repeat the entire run including specimen and control preparation, amplification and detection.

Interpretation of Results:

For a valid run, check each individual specimen for flags or comments on the result printout. Interpret the results as follows:

- A valid run may include both valid and invalid specimen results depending on whether flags and/or comments are obtained for the individual specimens.

Specimen results are interpreted as follows:

Control Results	Specimen Result	Flag Remark(s)	Interpretation
Invalid	Invalid	PC_INVALID and/or NC_INVALID	Control(s) not valid, all specimens in run marked invalid.
Positive for PC Negative for NC	(-)	No flag	<i>M. tuberculosis DNA not detected.</i> Specimen is presumptive negative for <i>M. tuberculosis</i> . A negative result does not preclude <i>M. tuberculosis</i> infection because results depend on adequate specimen collection, absence of inhibitors and sufficient DNA to be detected.
Positive for PC Negative for NC	MTB	No flag	<i>M. tuberculosis DNA detected.</i> Specimen is positive for <i>M. tuberculosis</i> .
Positive for PC Negative for NC	Invalid	S_INVALID	Specimen result is invalid. Right click on the Flag Remark to view the Detailed Flags. ⁽¹⁾
Positive for PC Negative for NC	Invalid	QS_INVALID	The Internal Control signal is abnormally high, the specimen is reported as Invalid. Process another aliquot of the original specimen and repeat the test. If the original specimen is not available, a new specimen must be collected. <u>Note:</u> AMPLILINK v3.3 software flags IC failures due to a high IC Ct as QS_INVALID.

(1) Refer to the “Flags” chapter in the “AMPLILINK software Version 3.3 Series Application Manual For use with COBAS® AmpliPrep instrument, COBAS® TaqMan® analyzer, COBAS® TaqMan® 48 analyzer, COBAS® AMPLICOR® analyzer, and cobas p 630 instrument,” for further information about Flag Remarks and Detailed Flags.

QUALITY CONTROL

One replicate of MYCO (-) Control and MTB (+) Control must be included in each test run. As with any new laboratory procedure, new operators should consider the use of additional controls each time the test is performed until a high degree of confidence is reached in their ability to perform the test correctly. There are no requirements regarding the position of the controls in the K-carrier. Check the run printout for flags and comments to ensure that the run is valid.

Negative Control

MYCO (-) C must yield a “**Target Not Detected**” result (if using AMPLILINK v3.2 software), or “**Negative**” (if using AMPLILINK v3.3 software) result, i.e., the Ct value for MTB DNA was above the limit for the assay or no Ct value for MTB DNA was obtained, but a valid Ct value was obtained for the Mycobacterium Internal Control DNA. If the **MYCO (-) C** does not meet this criteria, the entire run is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the **MYCO (-) C** is consistently not valid, contact your local Roche office for technical assistance.

Positive Control

For AMPLILINK v3.2 software, the assigned range for **MTB (+) C** is provided in the **COBAS® TaqMan® MTB Test Value Card**. The result for the **MTB (+) C** must fall within the range indicated in the **COBAS® TaqMan® MTB Test Value Card** for a run to be valid. The correct result will be displayed as “1 Positive”. If the positive control does not meet this criterion, then the entire run is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the result for the positive control is consistently outside the assigned range, contact your local Roche office for technical assistance.

For AMPLILINK v3.3 software, the assigned range for the **MTB (+) C** is set in the **Test Definition File** for the COBAS® TaqMan® MTB Test. The results for the **MTB (+) C** must fall within the assigned range to be valid. The correct result will be displayed as “**Positive**”. If the result for the **MTB (+) C** falls outside the assigned range, the result of the Positive Control will be **Invalid** with a **PC_INVALID** Flag Remark. If the Positive Control does not meet this criterion, then the entire run is invalid. Repeat the entire process (specimen and control preparation, amplification and detection). If the result for the Positive Control is consistently outside the assigned range, contact your local Roche office for technical assistance.

The MTB (+) Control contains approximately 20 copies/test of a *M. tuberculosis* plasmid DNA sequence. This is approximately four times the minimum detection level of the assay as determined by Poisson analysis.

Amplification and detection of the MTB (+) Control assures that amplification occurred. The MTB (+) Control will not monitor amplification efficiency or the detection level of the Test.

Internal Control

The Internal Control is intended to identify specimens that contain polymerase inhibitors. The use of the Internal Control will not eliminate all false negative test results.

PROCEDURAL PRECAUTIONS

As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.

PROCEDURAL LIMITATIONS

1. This test has only been validated using expectorated and induced sputum and BAL specimens that have been liquefied, decontaminated and concentrated using NALC-NaOH. Testing of other specimen types may result in false negative or false positive results.
2. Detection of *M. tuberculosis* is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., age, presence of symptoms), and/or stage of infection with *M. tuberculosis*.
3. False negative results may occur due to polymerase inhibition. The Mycobacterium Internal Control has been added to the COBAS® TaqMan® MTB Test to permit the identification of processed specimens containing substances that may interfere with PCR amplification of greater than 20 copies/test.
4. False negative results may occur in the presence of high titer Mycobacterium species other than *M. tuberculosis* (MTB) complex, if MTB is present at a lower titer than the interfering Mycobacterium species.
5. Reliable results are dependent on specimen collection, transport, storage and processing procedures. Variables due to storage have not been completely defined.
6. The addition of AmpErase enzyme to the Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Package Insert are necessary to avoid contamination of reagents.
7. Therapeutic success or failure cannot be determined using this test.
8. As with any diagnostic test, results from the COBAS® TaqMan® MTB Test should be interpreted considering all clinical and laboratory findings.
9. Use of this product should be limited only to personnel trained in the techniques of PCR.
10. The COBAS® TaqMan® MTB Test provides qualitative results. No correlation can be drawn between the magnitude of a positive COBAS® TaqMan® MTB Test elbow value and the number of *M. tuberculosis* cells within an infected specimen.
11. This product can only be used with the COBAS® TaqMan® 48 Analyzer.
12. The presence of PCR inhibitors may cause false negative or invalid results.
13. Though rare, mutations within the highly conserved region of the bacterial genome covered by the test's primers and/or probe may result in the failure to detect the bacteria.
14. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences.

NON-CLINICAL PERFORMANCE EVALUATION

Analytical Specificity

The analytical specificity of the COBAS® TaqMan® MTB Test was evaluated by testing the following bacteria, viruses and fungus, many of which are normal flora or pathogens common to the respiratory tract. None of the following organisms showed reactivity in the COBAS® TaqMan® MTB Test:

Mycobacterial Species

Mycobacterium abscessus
Mycobacterium asiaticum
Mycobacterium avium
Mycobacterium chelonae
Mycobacterium chitae
Mycobacterium fallax

Mycobacterium neoaurum
Mycobacterium nonchromogenicum
Mycobacterium phlei
Mycobacterium scrofulaceum
Mycobacterium senegalens
Mycobacterium shimoidei

Mycobacterium flavescens
Mycobacterium fortuitum
Mycobacterium gastri
Mycobacterium gordonae
Mycobacterium intracellulare
Mycobacterium kansasii
Mycobacterium komassense
Mycobacterium malmoense
Mycobacterium marinum

Mycobacterium simiae
Mycobacterium smegmatis
Mycobacterium sphagni
Mycobacterium szulgai
Mycobacterium terriae
Mycobacterium thermoresistibile
Mycobacterium triviale
Mycobacterium vaccae
Mycobacterium xenopi

Non-Mycobacterial Species

Acinetobacter calcoaceticus
Actinomadura madurae
Actinomyces pyogenes
Actinoplanes italicus
Aeromonas hydrophila
Arthrobacter oxydans
Bacillus subtilis
Bacteriodes fragilis
Blastomyces dermatitidis
Bordetella parapertussis
Bordetella pertussis
Branhamella (Moraxella) catarrhalis
Brevibacterium linens
Campylobacter jejuni
Candida albicans
Chlamydia trachomatis
Chromobacterium violaceum
Citrobacter freundii
Clostridium perfringens
Coccidioides immitis
Corynebacterium aquaticum
Corynebacterium diphtheriae
Corynebacterium flavescens
Corynebacterium glutamicum
Corynebacterium jeikeium
Corynebacterium minutissimum
Corynebacterium pseudodiphtheriticum
Corynebacterium pseudotuberculosis
Corynebacterium renale
Corynebacterium striatum
Neisseria meningitidis
Nocardia asteroides
Nocardia brasiliensis
Nocardia farcinica (W5218)
Nocardia nova (W5194)
Nocardia otitidiscaviarum
Nocardia transvalensis
Oerskovia turbata
Parainfluenza 2 virus
Peptococcus niger
Peptostreptococcus anaerobius
Peptostreptococcus magnus
Porphyromonas asaccharolytica
Porphyromonas gingivalis
Prevotella melaninogenica
Propionibacterium acnes
Proteus mirabilis
Pseudomonas aeruginosa

Corynebacterium xerosis
Cryptococcus neoformans
Deinococcus radiodurans
Dermatophilus congolensis
Derxia gummosa
Eikenella corrodens
Enterobacter aerogenes
Enterobacter cloacae
Enterococcus faecalis
Enterococcus faecium
Escherichia coli
Fusobacterium nucleatum
Gordona sputi
Haemophilus influenzae
Herpes simplex virus type 1
Histoplasma capsulatum
Human adenovirus
Human cytomegalovirus
Human enterovirus
Human rhinovirus 14
Influenza virus B
Klebsiella pneumoniae
Klebsiella pneumoniae subsp. ozaenae
Lactobacillus casei
Legionella micdadei
Legionella pneumophila
Mycoplasma hominis
Mycoplasma pneumoniae
Neisseria gonorrhoeae
Neisseria lactamica
Respiratory syncytial virus
Rhodococcus aichiensis
Rhodococcus chubuensis
Rhodococcus equi
Salmonellacholeraesuis subsp Choleraesuis
Serratia marcescens
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus gordonii
Streptococcus pneumoniae
Streptococcus pyogenes
Streptomyces griseinus
Veillonella atypica
Veillonella parvula
Vibrio parahaemolyticus
Xanthomonas maltophilia
Yersinia enterocolitica

Analytical Sensitivity: *M. tuberculosis* Cells

The limit of detection of the COBAS® TaqMan® MTB Test was established by determining the minimum number of *M. tuberculosis* colony-forming units (CFU) that can be reproducibly detected (≥ 95% Positive Results) by the COBAS® TaqMan® MTB Test. A decontaminated culture of MTB was diluted into

NALC/NaOH-liquefied negative sputum to produce a panel of 7 levels containing 10, 5, 1, 0.5, 0.25, 0.1 and 0 MTB CFU/PCR. This panel was prepared independently each day for three days. Fifty-four replicates of each panel level were tested using three lots of COBAS® TaqMan® MTB Test reagents. Results from Probit analysis (Table 1) indicate the limit of detection for the COBAS® TaqMan® MTB Test to be 0.46 CFU/PCR or approximately 18 CFU/mL in sputum specimens.

Table 1
Limit of Detection of the COBAS® TaqMan® MTB Test with *M. tuberculosis* Cells

Member	Nominal Input CFU/PCR	# Invalid	# Valid	# Pos	# Trials	% Hits	95% CI
1	10	0	54	54	54	100.0%	94.6 - 100%
2	5	0	54	54	54	100.0%	94.6 - 100%
3	1	0	54	54	54	100.0%	94.6 - 100%
4	0.5	0	54	51	54	94.4%	84.6 - 98.8%
5	0.25	0	54	46	54	85.2%	72.9 - 93.4%
6	0.1	0	54	33	54	61.1%	46.9 - 74.0%
7	0	0	54	0	54	0.0%	0.0 - 5.4%

95% LOD by PROBIT = 0.46 CFU/PCR	95% Confidence Interval = 0.33 - 0.83 CFU/PCR
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Inclusivity

The COBAS® TaqMan® MTB Test was evaluated with all species of Mycobacteria found in the MTB Complex (*M. tuberculosis*, *M. bovis*, *M. microti*, and *M. africanum*). Quantified genomic DNA preparations (10 copies/PCR) and bacterial cultures were examined. At the input level of 10 genomic copies/PCR, all 4 members of the MTB Complex were identified as positive. In addition, 169 cultures of MTB Complex species (*M. tuberculosis* = 159, *M. bovis* = 6, *M. microti* = 3, and *M. africanum* = 1) were analyzed with the COBAS® TaqMan® MTB Test. One culture of *M. microti* and one culture of *M. tuberculosis* tested negative, giving an overall inclusivity rate of 99%.

Reproducibility

The COBAS® TaqMan® MTB Test was evaluated for reproducibility through analysis of day to day, lot to lot and operator to operator results. The day to day study (Table 2) was run by one operator with one COBAS® TaqMan® MTB Test reagent lot for 15 days using the MYCO Negative Control (NC), MTB Positive Control (PC) and MTB Positive Control diluted with Negative Control (1/2 PC). The lot to lot study (Table 3) was run by one operator in one day with three lots of COBAS® TaqMan® MTB Test reagents using the MYCO Negative Control (NC), MTB Positive Control (PC) and MTB Positive Control diluted with Negative Control (1/4 PC and 1/2 PC). The operator to operator study (Table 4) was run by three operators in three days with one lot of COBAS® TaqMan® MTB Test reagents using MYCO Negative Control (NC), MTB Positive Control (PC) and MTB Positive Control diluted with Negative Control (1/2 PC).

The 720 results from these three studies are summarized in Tables 2-4. Negative results were obtained with 100% of the 132 NC replicates tested. All of the 588 positive replicates, ranging from 20 to 5 copies of MTB target/PCR tested positive. From all three studies, the %CV was 2.4 or less, demonstrating good reproducibility of qualitative results with this test.

Table 2
Day to Day Reproducibility Results for the COBAS® TaqMan® MTB Test

MTB Target	NC (0 c/PCR)	PC (~20 c/PCR)	1/2 PC (~10 c/PCR)
Total Number of Valid Replicates	60	60	60
% Positive Results	0%	100%	100%
Mean MTB Elbow	N/A	39.4	41.1
Minimum Value	N/A	38.3	39.9
Maximum Value	N/A	40.8	42.8
Standard Deviation	N/A	0.5	0.7
Coefficient of Variation	N/A	1.3%	1.7%

Table 3
Lot to Lot Reproducibility Results for the COBAS® TaqMan® MTB Test

MTB Target	NC (0 c/PCR)	PC (~20 c/PCR)	1/2 PC (~10 c/PCR)	1/4 PC (~5 c/PCR)
Total Number of Valid Replicates	36	36	36	324
% Positive Results	0%	100%	100%	100%
Mean MTB Elbow	N/A	39.6	40.7	41.6
Minimum Value	N/A	38.5	39.1	39.5
Maximum Value	N/A	41.3	42.3	48.0
Standard Deviation	N/A	0.8	0.7	1.0
Coefficient of Variation	N/A	1.9%	1.7%	2.4%

Table 4
Operator to Operator Reproducibility Results for the COBAS® TaqMan® MTB Test

MTB Target	NC (0 c/PCR)	PC (~20 c/PCR)	1/2 PC (~10 c/PCR)
Total Number of Valid Replicates	36	36	36
% Positive Results	0%	100%	100%
Mean MTB Elbow	N/A	39.6	40.8
Minimum Value	N/A	38.4	39.2
Maximum Value	N/A	41.5	42.6
Standard Deviation	N/A	0.8	0.8
Coefficient of Variation	N/A	1.9%	2.0%

Correlation

A correlation study was executed to compare the performance of the COBAS® TaqMan® MTB Test with the COBAS® AMPLICOR® MTB Test. A total of 769 sputum specimens and 186 bronchial alveolar lavage (BAL) specimens were analyzed in the study. Specimens were obtained either from frozen archives or from freshly collected frozen retains. Three unique lots of COBAS® TaqMan® MTB Test reagents were used in the study.

The positive correlation rate for sputum specimens (Table 5) was 96.2% and the negative correlation rate was 99.1%, resulting in a total agreement of 98.3% for sputum specimens between the COBAS® TaqMan® MTB Test and the COBAS® AMPLICOR® MTB Test. The positive correlation rate for BAL specimens (Table 6) was 97.1% and the negative correlation rate was 99.3%, resulting in an over all agreement of 98.9% for BAL specimens between the COBAS® TaqMan® MTB Test and the COBAS® AMPLICOR® MTB Test.

Table 5
Correlation between COBAS® TaqMan® MTB Test and COBAS® AMPLICOR® MTB Test
Total Sputum Results

Sputum Results N = 769		COBAS® AMPLICOR® MTB Test		Total
		+	-	
COBAS® TaqMan® MTB Test	+	207	5	212
	-	8	549	557
Total		215	554	769

	Ratio	% Agreement	95% Confidence Interval
Positive Percent Agreement	207/215	96.2%	92.8 - 98.4
Negative Percent Agreement	549/554	99.1%	97.9 - 99.7
Total Agreement	756/769	98.3%	97.1 - 99.1

Table 6
Correlation between COBAS® TaqMan® MTB Test and COBAS® AMPLICOR® MTB Test
Total BAL Results

BAL Results N = 186		COBAS® AMPLICOR® MTB Test		Total
		+	-	
COBAS® TaqMan® MTB Test	+	34	1	35
	-	1	150	151
Total		35	151	186

	Ratio	% Agreement	95% Confidence Interval
Positive Percent Agreement	34/35	97.1%	85.1 - 99.9
Negative Percent Agreement	150/151	99.3%	96.4 - 99.9
Total Agreement	184/186	98.9%	98.9 - 99.9

Whole System Failure

The Whole System Failure rate was determined for the COBAS® TaqMan® MTB Test by examining the percent positive results with 100 replicates of negative sputum spiked with MTB culture to a final concentration of 60 CFU/mL or ~ 3 times the limit of detection of the test (see Limit of Detection section above). Positive results were obtained for 99 of the 100 replicates, yielding a Whole System Failure rate of 1.0% and a system success rate of 99% for the COBAS® TaqMan® MTB Test.

Interference

The COBAS® TaqMan® MTB Test was evaluated for endogenous hemoglobin interference by testing negative sputum spiked with MTB Positive Control (25 Copies/PCR) and hemoglobin from 0.52 to 5.2 mg/mL. There was no interference with the COBAS® TaqMan® MTB Test at all levels of spiked hemoglobin.

The COBAS® TaqMan® MTB Test was evaluated for interference by six anti-tuberculosis drugs commonly used in therapy. The six drugs (Table 7) were tested at 1 x C_{Max} and 3 x C_{Max} by spiking drugs into negative sputum containing MTB Positive Control plasmid (~17 Copies/PCR). There was no effect on COBAS® TaqMan® MTB Test performance with any of the six drugs spiked at both 1 x C_{Max} and 3 x C_{Max} levels.

Table 7
Anti-tuberculosis Drugs and C_{Max} Levels

Drug	Chemical Name	Abbreviation	C _{Max} (µg/mL)
Isoniazid	Isonicotinic acid hydrazide	INH	5 - 7
Ethambutol	Ethambutol dihydrochloride	ETH	1.7
Rifampicin	Rifadin Rimactane	RIF	7.99
Pyrazinamide	Pyrazinecarboxamide	PZA	30 - 35
Kanamycin	Kanamycin monosulfate	KM	2.0
Streptomycin	Streptomycin sulfate salt	SM	25 - 30

Inhibition

The inhibition rate (invalid Internal Control) for the COBAS® TaqMan® MTB Test was determined through analysis of several studies using sputum and BAL specimens as well as MTB culture panel materials, totaling 1094 data points. The overall inhibition rate for the COBAS® TaqMan® MTB Test for all studies analyzed (specimens and MTB culture panel materials) was 0.0% (0/1094).

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Document Revision Information	
Doc Rev. 4.0 5/2010	<p>Harmonization of the terminology for describing extracted specimens prior to Working Master Mix addition; extracted specimens prior to MMX addition will now only be referred to as Prepared Specimens throughout the package insert.</p> <p>The storage parameters for storing Prepared Specimens will be updated to accurately describe the proper storage parameters. This revised text will indicate: "Prepared specimens are stable for up to 4 hours at room temperature or up to 4 days at 2-8°C before addition into Working Master Mix."</p>
Doc Rev. 3.1 11/2009	<p>Under the <i>NON-CLINICAL PERFORMANCE EVALUATION - Correlation</i> section, the location of the data has been corrected for Tables 5 and 6.</p> <p>Please contact your local Roche Representative if you have any questions.</p>



COBAS® TaqMan® MTB Test manufactured by:

Roche Molecular Systems, Inc., Branchburg, NJ, 08876 USA
A Member of the Roche Group



Distributed by

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877 273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

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Roche Diagnostics GmbH
D-68298 Mannheim

