cobas® MAI

Nucleic acid test
for use on the cobas® 6800/8800 Systems

For in vitro diagnostic use

cobas® MAI	P/N: 08412138190
cobas® MAI Positive Control Kit	P/N: 07544863190
cobas® 6800/8800 Buffer Negative Control Kit	P/N: 07002238190
cobas® Microbial Inactivation Solution (MIS)	P/N: 08185476001

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Intended use

cobas® MAI for use on the **cobas®** 6800/8800 Systems is an automated, qualitative *in vitro* diagnostic test, that utilizes real-time polymerase chain reaction (PCR), for the direct detection and differentiation of *Mycobacterium avium* and *Mycobacterium intracellulare* DNA in human respiratory specimens including raw sputum, and digested and decontaminated (N-acetyl-L-cysteine/NaOH [NALC-NaOH]-treated) sputum and bronchoalveolar lavage (BAL) samples. This test is intended for use in conjunction with culture as an aid in the diagnosis of *M. avium-intracellulare* complex (MAC) infection.

Summary and explanation of the test

Background

Mycobacterium avium and *M. intracellulare* are two closely related, distinct species of nontuberculous mycobacteria (NTM), which comprise the MAC, and may also be grouped together as *M. avium intracellulare* (MAI). NTM are mycobacterial species other than *M. tuberculosis* and *M. leprae*. NTM are generally free living organisms that are ubiquitous in the environment.[1-4] They have been recovered from surface water, tap water, soil, domestic and wild animals, milk, and food products. Although NTM can colonize body surfaces and secretions without causing disease, they have been associated with four distinct clinical syndromes; progressive pulmonary disease (MAC, *M. kansasii* and *M. abscessus*), superficial lymphadenitis, seen commonly in pediatric populations, (MAC, *M. scrofulaceum*, *M. malmoense*), disseminated disease in severely immunocompromised patients, and skin and soft tissue infection usually as a consequence of direct inoculation.[5]

MAC currently comprises twelve species of environmental and animal associated, slowly growing mycobacteria: *M. avium*, *M. intracellulare*, *M. chimaera*, *M. colombiense*, *M. arosiense*, *M. bouchedurhonense*, *M. marseillense*, *M. timonense*, *M. indicus pranii*, *M mantenii*, *M. vulneris*, *M. yongonense*.[6,7] There are 28 serovars of *M. avium* and *M. intracellulare*[8] and *M. avium* consists of 4 subspecies, *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *hominissuis*, and *M. avium* subsp. *silvaticum*.[6] MAC is the NTM most commonly associated with human disease.[5]

MAC is primarily a pulmonary pathogen that affects individuals who are immune compromised (e.g., patients with AIDS, hairy cell leukemia, or on immunosuppressive chemotherapy). MAC is transmitted via inhalation into the respiratory tract and ingestion into the GI tract. There is no evidence of animal-human or human-human transmission and it is thus considered non communicable. Pulmonary MAC infections are associated with chronic lung diseases, such as COPD, chronic bronchitis, bronchiectasis, cystic fibrosis, and lung cancer. MAC may also cause osteomyelitis, tenosynovitis, synovitis, and disseminated MAC (DMAC) can involve lymph nodes, CNS, liver, spleen and bone marrow. Cutaneous infections occur usually through direct inoculation. MAC is the most common cause of infection by NTM in patients with AIDS. *M. avium* accounts for more than 95% of patients with AIDS who develop MAC infections. MAC lung disease occurs rarely in immunocompetent hosts. *M. intracellulare* is responsible for 40% of infections in immunocompetent patients. Lady Windermere syndrome refers to a pattern of pulmonary MAC infection believed to be associated with suppression of cough in otherwise healthy, thin, elderly women.

The incidence of NTM disease is difficult to ascertain because NTM are considered non communicable and are therefore not reportable to public health agencies in many countries. Incidence rate estimates are based on the number of NTM isolates reported and appear to be similar in most developed countries ranging from 1.0 to 1.8 cases per 100,000 persons.[5,9] In 2009, a study in Oregon estimated an annualized rate of 5.6 cases of MAC pulmonary infection per 100,000's population,

with most cases (60%) affecting females.[10] The highest number of DMAC cases reported in the United States was 37,000 in 1994, at the peak in the AIDS epidemic, and the incidence has declined since the adoption of highly active antiretroviral therapy. A surveillance study estimated the incidence of NTM pulmonary infections in patients without HIV infection was 0.72-0.74 per 100,000 inhabitants in France from 2001-2003.[11] And in 2004, a similar study in New Zealand estimated the incidence of NTM disease at 1.92 per 100,000's population.[12]

The diagnosis of pulmonary MAC infection should be considered in symptomatic patients presenting with nodular or cavitary opacities on chest radiograph, or a high resolution CT scan that shows multifocal bronchiectasis with multiple small nodules, when infection with MTB and other appropriate diagnoses have been excluded.[5] AFB smear and culture is recommended for diagnosis. Diagnosis requires:

(i) positive cultures from at least two separate expectorated sputum samples, or

(ii) positive culture results from at least one bronchial wash or lavage, or

(iii) transbronchial or other lung biopsy with mycobacterial histopathologic features, and positive culture from biopsy, and one or more sputum or bronchial washings that are culture positive for MAC confirms infection.[5]

NTM, including MAC, should be identified to species level. Treatment involves 2 or 3 first line antimicrobials for 12 months. The first line regimen includes macrolides (clarithromycin or azithromycin), ethambutol, and rifamycins (rifampin); and the second line antimicrobial regimen includes the aminoglycosides (streptomycin or amikacin). Routine susceptibility testing of MAC isolates is recommended for clarithromycin only due to poor correlation between in vitro results and clinical outcomes.[5]

The diagnosis of MAC infection can be established based on both clinical presentation and radiographic findings, and confirmed by recovery of the organism in culture as described above[5] but culture is slow and can take days to weeks. Alternatively, nucleic acid amplification tests can detect and differentiate *M. avium* and *M. intracellulare* directly from clinical samples in hours for a more rapid diagnosis and initiation of empiric therapy. However, drug susceptibility testing (DST) is required to confirm efficacy of empiric therapy and requires subculture of clinical isolates and may take days to weeks for results depending on the method.

Explanation of the test

cobas® MAI for use on the **cobas®** 6800/8800 Systems (referred to as **cobas®** MAI throughout the remainder of this document) is an automated, qualitative real-time PCR test designed to detect and differentiate *Mycobacterium avium* and *Mycobacterium intracellulare* DNA in human respiratory specimens including raw sputum specimens; and digested and decontaminated NALC-NaOH-treated sputum and BAL sediments. The DNA Internal Control, used to monitor the entire sample preparation and PCR amplification process, is introduced into each specimen during sample processing on the **cobas®** 6800/8800 Systems. In addition, the test utilizes a low titer positive and a negative control.

Principles of the procedure

cobas® MAI is based on pre-analytic sample liquefaction and mycobacteria inactivation followed by sample sonication and fully automated sample preparation (nucleic acid extraction and purification) and PCR amplification and detection. Sample liquefaction and mycobacteria inactivation occur simultaneously during sample incubation with **cobas®** Microbial Inactivation Solution (MIS). Sonication of liquefied and inactivated sample is performed prior to loading onto the **cobas®** 6800/8800 Systems. The **cobas®** 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas®** 6800/8800 software

which assigns test results for all tests as positive, negative or invalid. Results can be reviewed directly on the system screen, exported, or printed as a report.

Nucleic acid from patient samples, external controls and added internal control DNA (DNA-IC) molecules is simultaneously extracted. In summary, bacterial nucleic acid is released by chemical (**cobas**® Microbial Inactivation Solution [MIS], **cobas omni** Lysis Reagent), enzymatic (proteinase) and physical (sonication) disruption of bacteria. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris and potential PCR inhibitors are removed with subsequent wash steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the sample is achieved by the use of target-specific forward and reverse primer for the *M. avium* complex which is selected from a highly-conserved region within the respective target organism. MAC is detected by one selective set of primers and *M. avium* and *M. intracellulare* are differentiated by two distinct probes within the amplification region (16S rRNA gene). Selective amplification of DNA IC is achieved by the use of sequence-specific forward and reverse primers which are selected to have no homology with the *M. avium* complex target region. A thermostable DNA polymerase enzyme is used for PCR amplification. The target and DNA-IC sequences are amplified simultaneously utilizing a universal PCR amplification profile with predefined temperature steps and number of cycles. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon). Any contaminating amplicon from previous PCR runs are eliminated by the AmpErase enzyme, which is included in the PCR master mix, during the first thermal cycling step.[13] However, newly formed amplicons are not eliminated since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® MAI master mix contains one detection probe each for *M. avium* and *M. intracellulare* and one for the DNA-IC. The target specific probes are labeled with different fluorescent reporter dyes allowing simultaneous detection of *M. avium* target, *M. intracellulare* target and DNA-IC in three different target channels.[14,15] When not bound to the target sequence, the fluorescent signal of the intact probes is suppressed by a quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage of the probe by the 5' to 3' exonuclease activity of the DNA polymerase causing the separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye increases concomitantly. Real-time detection and discrimination of PCR products is accomplished by measuring the fluorescence of the released reporter dyes for the *M. avium* complex targets and DNA-IC, respectively.

Reagents and materials

cobas® MAI reagents and controls

All unopened reagents and controls must be stored as recommended in Table 1 to Table 5.

Table 1 cobas® MAI

cobas® MAI Store at 2-8°C 384 test cassette (P/N 08412138190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, Calcium chloride, Calcium acetate, 8% Proteinase EUH210: Safety data sheet available on request. EUH208: Contains Subtilisin from *Bacillus subtilis*. May produce an allergic reaction.	38 mL
DNA Internal Control (DNA-IC)	Tris buffer, < 0.05% EDTA, < 0.001% non-MAC related DNA construct, 0.002% Poly rA RNA (synthetic), < 0.1% Sodium azide	38 mL
Elution Buffer (EB)	Tris buffer, 0.2% Methyl-4 hydroxibenzoate	38 mL
Master Mix Reagent 1 (MMX-R1)	Manganese acetate, Potassium hydroxide, < 0.1% Sodium azide	14.5 mL
MAI Master Mix Reagent 2 (MAI MMX-R2)	Tricine buffer, Potassium acetate, EDTA, Glycerol, < 18% Dimethyl sulfoxide, < 0.12% dATP, dCTP, dGTP, dUTPs, < 0.1% Tween 20, < 0.1% Sodium azide, < 0.1% Z05 DNA polymerase, < 0.1% AmpErase (uracil-N glycosylase) enzyme (microbial), < 0.01% Internal Control forward and reverse primers, < 0.01% Upstream and downstream MAI primers, < 0.01% Fluorescent-labeled oligonucleotide probes specific for MAI and the DNA Internal Control, < 0.01% Oligonucleotide aptamer	17.5 mL

Table 2 cobas® MAI Positive Control Kit

cobas® MAI Positive Control Kit Store at 2-8°C (P/N 07544863190)		
Kit components	**Reagent ingredients**	**Quantity per kit**
MAI Positive Control (MAI (+) C)	Tris buffer, < 0.05% Sodium azide, < 0.05% EDTA, < 0.002% Poly rA, < 0.01% Non-infectious plasmid DNA (microbial) containing *M. avium* and *M. intracellulare* genomic sequences	16 mL (16 x 1 mL)

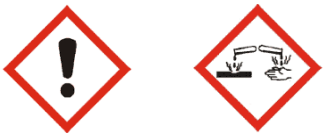
Table 3 **cobas**® 6800/8800 Buffer Negative Control Kit

cobas® 6800/8800 Buffer Negative Control Kit
Store at 2-8°C
(P/N 07002238190)

Kit components	Reagent ingredients	Quantity per kit
cobas® 6800/8800 Buffer Negative Control (BUF (-) C)	Tris buffer, < 0.1% sodium azide, EDTA, < 0.002% Poly rA RNA (synthetic)	16 mL (16 x 1 mL)

cobas omni reagents for sample preparation

Table 4 cobas omni reagents for sample preparation*

Reagents	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas omni MGP Reagent (MGP) Store at 2-8°C (P/N 06997546190)	Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	480 tests	Not applicable
cobas omni Specimen Diluent (SPEC DIL) Store at 2-8°C (P/N 06997511190)	Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide	4 x 875 mL	Not applicable
cobas omni Lysis Reagent (LYS) Store at 2-8°C (P/N 06997538190)	43% (w/w) guanidine thiocyanate, 5% (w/v) polydocanol, 2% (w/v) dithiothreitol, dihydro sodium citrate	4 x 875 mL	 **DANGER** H302 + H332: Harmful if swallowed or if inhaled. H314: Causes severe skin burns and eye damage. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.
cobas omni Wash Reagent (WASH) Store at 15-30°C (P/N 06997503190)	Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate	4.2 L	Not applicable

* These reagents are not included in the **cobas**® MAI kit. See listing of additional materials required (Table 10).

**Product safety labeling primarily follows EU GHS guidance

cobas® Microbial Inactivation Solution

Table 5 **cobas**® Microbial Inactivation Solution*

cobas® Microbial Inactivation Solution
Store at 2-8°C
(P/N 08185476001)

Kit components	Reagent ingredients	Quantity per kit	Safety symbol and warning**
cobas® Microbial Inactivation Solution (MIS)	Tris buffer, 60% (v/v) Isopropanol, 1% (w/v) Thymol, 18.9% (w/v) Guanidinium thiocyanate, 1.4% (w/v) Tris(2-carboxyethyl)-phosphine hydrochloride, 0.4% (w/v) Tween 20	480 mL (16 x 30 mL)	 **DANGER** H225: Highly flammable liquid and vapour. H314: Causes severe skin burns and eye damage. H336: May cause drowsiness or dizziness. H412: Harmful to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P280: Wear protective gloves/ protective clothing/ eye protection/ face protection. P303 + P361 + P353: IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor. P370 + P378: In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish.

* This reagent is not included in the **cobas**® MAI kit.

**Product safety labeling primarily follows EU GHS guidance

Reagent storage and handling requirements

Reagents must be stored and handled as specified in Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 6.

Table 6 Reagent storage (when reagent is not on the system)

Reagent	Storage temperature
cobas® MAI	2-8°C
cobas® MAI Positive Control Kit	2-8°C
cobas® Buffer Negative Control Kit	2-8°C
cobas omni Lysis Reagent	2-8°C
cobas omni MGP Reagent	2-8°C
cobas omni Specimen Diluent	2-8°C
cobas omni Wash Reagent	15-30°C (room temperature)

Once loaded onto the **cobas**® 6800/8800 Systems reagents are automatically stored at appropriate temperatures and their expiration is monitored by the system. The **cobas**® 6800/8800 Systems allow reagents to be used only if all of the conditions shown in Table 7 are met. The system automatically prevents use of expired reagents. Table 7 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

Table 7 Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

Reagent	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® MAI	90 days from first usage	Max 40 runs	Max 40 hours
cobas® MAI Positive Control Kit	Not applicable	Not applicable	Max 10 hours
cobas® Buffer Negative Control Kit	Not applicable	Not applicable	Max 10 hours
cobas omni Lysis Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni MGP Reagent	30 days from loading*	Not applicable	Not applicable
cobas omni Specimen Diluent	30 days from loading*	Not applicable	Not applicable
cobas omni Wash Reagent	30 days from loading*	Not applicable	Not applicable

*Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

Store MIS at the temperature specified in Table 8.

Table 8 cobas® Microbial Inactivation Solution storage

Reagent	Storage temperature
MIS	2-8°C

Unopened MIS is stable until the expiration date indicated. Once opened, this reagent is stable for 30 days when stored at 2-8°C including cumulative 5 hours at 15-30°C (room temperature) or until expiration date, whichever comes first, as specified in Table 9.

Table 9 cobas® Microbial Inactivation Solution expiry conditions

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	Stability at 15-30°C (room temperature)
MIS	Date not passed	30 days from first usage	Not applicable	Max 5 hours

Additional materials required

Table 10 Materials and consumables for use on **cobas**® 6800/8800 Systems

Material	P/N
cobas omni Processing Plate	05534917001
cobas omni Amplification Plate	05534941001
cobas omni Pipette Tips	05534925001
cobas omni Liquid Waste Container	07094388001
cobas omni Lysis Reagent	06997538190
cobas omni MGP Reagent	06997546190
cobas omni Specimen Diluent	06997511190
cobas omni Wash Reagent	06997503190
Solid Waste Bag and Solid Waste Container or Solid Waste Bag With Insert and Kit Drawer Solid Waste Update	07435967001 and 07094361001 or 08030073001 and 08387281001
MPA RACK 13 MM LIGHT GREEN 7001-7050*	03118878001 or equivalent

* MPA 13mm racks are required to use **cobas**® MAI. Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

Table 11 Other materials and consumables required for pre-analytic workflow

Materials
Tube sonicator TS 5 (Rinco Ultrasonics AG - P/N 46690)
5 mL polypropylene screw cap tubes 75x13mm, round base (Sarstedt - Tube P/N 60.504.010, Screw cap P/N 65.163)*
MPA RACK 13 MM LIGHT GREEN 7001-7050 (Roche - P/N 03118878001 or equivalent)**
Centrifuge (Option to restrict RCF to max. 3000 x g, compatible with 75x13mm screw-cap tubes)
Vortex mixer
Thermostable barcode labels (OPAL Associates AG, P/N 20300824 TR PE-Folie Pharma or equivalent)***

* Use of tubes other than those recommended above must be verified by user prior to implementation into **cobas**® MAI workflow in the laboratory

** MPA 13mm racks are required to run the tube sonicator TS 5. Contact your local Roche representative for a detailed order list for equivalent sample racks in other colors or number ranges. Note that RD5 racks are not compatible with the tube sonicator TS 5.

***For further details on barcode specifications refer to the **cobas**® 6800/8800 Systems User Guide. Use of barcode labels other than those recommended above must be verified by user prior to implementation into **cobas**® MAI workflow in the laboratory. Contact your local Roche representative for further details on compatible barcode labels and suggestions for compatibility verification. The use of non-compatible barcode labels may lead to tube damage during sonication and subsequent contamination of instrument.

Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® MAI analysis package must be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

Table 12 Instrumentation

Equipment	**P/N**
cobas® 6800 System (Moveable Platform)	05524245001 and 06379672001
cobas® 6800 System (Fixed Platform)	05524245001 and 06379664001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001
Instrument Gateway	06349595001

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- All patient samples should be considered potentially infectious. Therefore, all biological specimens should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories, in the CLSI Document M29-A4 and in the Tuberculosis Laboratory Biosafety Manual by WHO.[16,17,18] Only personnel proficient in handling infectious materials and the use of **cobas**® MAI and **cobas**® 6800/8800 Systems should perform this procedure.
- All personnel should wear protective personal equipment, including laboratory coats, disposable gloves, and eye and respiratory protection according to their institutions safety procedures and practices and should follow their institution's safety procedures for working with chemicals and biological specimens.
- Specimen inactivation by MIS should be performed in a biological safety cabinet (BSC; Type A2) within a Biosafety Level 3[19] laboratory or other biosafety control environment according to local and institutional guidelines or regulations.
- Success in mycobacterial inactivation depends on adherence to procedures outlined in this document and complete mixing of sample with MIS. Pre-analytic treatment of patient samples by MIS reduces, but may not completely eliminate, the risk of mycobacterial infection.
- If spillage of samples in MIS (which contains guanidinium thiocyanate) occurs, do not allow it to come in contact with sodium hypochlorite containing disinfectants such as bleach. This mixture can produce a highly toxic gas.
- MIS is light-sensitive and shipped in light-protective bottles. MIS must be stored upright.
- Use only supplied or specified required consumables to ensure established test performance.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect established test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.

Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples, reagents, or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent and MIS contain guanidinium thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- Do not allow **cobas omni** Lysis Reagent or MIS, which contain guanidinium thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Expended control kits contain pierced vials with residual reagent; special care should be taken during disposal to avoid spills and contact.

- **cobas**® MAI kit, **cobas**® MAI Positive Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Sample inactivation by MIS should be performed in a biological safety cabinet (BSC; Type A2) within a Biosafety Level 3[19] laboratory or other biosafety control environment according to local and institutional guidelines or regulations.
- Wear laboratory gloves, laboratory coats, and eye and respiratory protection when handling samples and reagents according to institutional guidelines. Avoid contaminating gloves when handling samples and controls. Gloves must be changed between handling samples and **cobas**® MAI kit, **cobas**® MAI Positive Control kit, **cobas**® 6800/8800 Buffer Negative Control kit**,** and **cobas omni** reagents to prevent contamination.
- Disinfect and wash hands thoroughly after handling samples and reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 Systems, follow the instructions in the **cobas**® 6800/8800 Systems User Guide to properly clean and decontaminate the surface(s) of instrument(s).

Specimen collection, transport, and storage

Note: Handle all samples and controls as if they are capable of transmitting infectious agents.

Specimens

Raw sputum and NALC-NaOH-treated sputum and BAL sediments may be used with **cobas**® MAI.

Specimen transport and storage

Raw sputum specimens may be stored and/or transported for up to 3 days at 2°C to 35°C, followed by up to 7 days at 2°C to 8°C prior to sample liquefaction and inactivation by MIS. For long-term storage of MIS untreated raw sputum specimens, temperatures at ≤ -20°C are recommended.

NALC-NaOH-treated sputum and BAL sediment specimens may be stored for up to 7 days at 2°C to 8°C prior to sample inactivation by MIS. For long-term storage of MIS untreated sputum and BAL sediments, specimens may be stored frozen at temperatures ≤ -20°C for up to 9 months including two freeze/thaw cycles.

If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of infectious samples and etiologic agents.

Inactivated specimen storage

Raw sputum and NALC-NaOH-treated sputum and BAL sediment specimens treated with MIS (inactivated) may be stored for up to 12 hours at 15°C to 35°C, followed by up to 7 days at 2°C to 8°C and 30 days at ≤ -20°C including two freeze/thaw cycles prior to processing on the **cobas**® 6800/8800 Systems.

Note: MIS-treated specimens may not freeze due to high isopropanol content.

Note: Sonication of specimens may be performed at any time after an initial incubation with MIS for a minimum of 60 minutes. Refer to the "Sonication of specimens" section for more details.

Instructions for use

Procedural notes

- Do not use **cobas**® MAI, **cobas**® MAI Positive Control Kit, **cobas**® Buffer Negative Control Kit, MIS or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Ensure that thermostable barcode labels on sample tubes are oriented towards and visible through the slits open at the top on the side of MPA sample racks. Refer to Figure 1 and to the **cobas**® 6800/8800 Systems User Guide for proper barcode specifications and additional information on loading sample tubes.
- Ensure that sample tubes are uncapped after sonication and before loading on the **cobas**® 6800/8800 Systems.
- Refer to the **cobas**® 6800/8800 Systems User Guide for proper maintenance of instruments.

Prior to running MAI on the **cobas**® 6800/8800 Systems, specimens must be processed according to the following sections: "Processing of raw sputum specimens" or "Processing of sputum and BAL sediments" and "Sonication of specimen". Abbreviated representative workflows are summarized in Table 13 for the raw sputum specimen type and in Table 14 for the sediment specimen type. For further details, refer to the subsequent sections.

Note: Specimen inactivation by MIS should be performed under a biological safety cabinet (BSC; Type A2) within a Biosafety Level 3[19] laboratory or other biosafety measures according to local and institutional guidelines or regulations.

Note: Sonication of MIS-treated specimens may be performed within a BSL-2 laboratory or other biosafety controlled environment according to local and institutional guidelines or regulations.

Table 13 Workflow overview - Raw sputum specimen type

BSL-3 (BSC Type A2)	1			Add 2 parts of MIS to 1 part of raw sputum
	2		30-60 seconds	Shake vigorously or vortex for 30-60 seconds
	3		≥ 60 minutes	Incubate sample for at least 60 min at 15-30°C (room temperature)
	4		30-60 seconds	Shake vigorously or vortex for 30-60 seconds
	5		1.2 mL for 1 test 2.4 mL for 2 tests 3.6 mL for 3 tests	Transfer 1.2 to 3.6 mL of MIS-treated sample to screw cap secondary tube
BSL-2	6		5 minutes	Sonicate MIS-treated sample
	7		Max. 1 minute	Centrifuge sample for no more than 1 minute at maximal RCF of 3000 x g
	8			Load uncapped sample on **cobas**® 6800/8800 Systems and start run using the raw sputum specimen type

Table 14 Workflow overview - Sediment specimen type

BSL-3 (BSC; Type A2)	1		0.2 mL for 1 test 0.4 mL for 2 tests 0.6 mL for 3 tests	Vortex and transfer 0.2 to 0.6 mL of sediment sample to screw cap secondary tube
	2			Add 5 parts of MIS to 1 part of sediment sample • 1 mL MIS for 1 test (0.2 mL sediment sample) • 2 mL MIS for 2 tests (0.4 mL sediment sample) • 3 mL MIS for 3 tests (0.6 mL sediment sample)
	3		30-60 seconds	Shake vigorously or vortex for 30-60 seconds
	4		≥ 60 minutes	Incubate sample for at least 60 minutes at 15-30°C (room temperature)
	5		30-60 seconds	Shake vigorously or vortex for 30-60 seconds
BSL-2	6		5 minutes	Sonicate MIS-treated sample
	7		Max. 1 minute	Centrifuge sample for no more than 1 minute at maximal RCF of 3000 x g
	8			Load uncapped sample on **cobas**® 6800/8800 Systems and start run using the sediment specimen type

Processing of raw sputum specimens

- Confirm that the raw sputum container is properly labeled and contains a minimum of 0.4 mL of sputum. If stored frozen, thaw and equilibrate sample to ambient temperature.
- Invert the MIS bottles two to four times before use.
- Open the sputum container and add approximately two parts of MIS to one part of sputum specimen (e.g., 2 mL of MIS to 1 mL of sputum specimen) by visual volume estimation and using a disposable pipette. Close the sputum container tightly.
- Close the MIS bottles immediately after use.
- Shake vigorously or vortex for 30-60 seconds.

  Note: Ensure that the entire sputum specimen is mixed with MIS.

- Incubate specimen for at least 60 minutes at 15-30°C (room temperature).

  Note: Refer to the "Inactivated specimen storage" section for maximal storage conditions.

- Shake vigorously or vortex for 30-60 seconds or until sample is fully homogenized.
- Transfer a minimum of 1.2 mL and no more than 3.6 mL of MIS treated sputum specimen into a thermostable barcode labeled 5 mL polypropylene screw-cap tube 75x13mm, round base (Sarstedt - Tube P/N 60.504.010, Cap P/N 65.163). Firmly close the tube.

  Note: Prior to specimen transfer confirm that barcode information on the sputum container and the 5 mL secondary tube match.

  Note: Refer to Table 15.

- Sonicate inactivated specimen according to section "Sonication of specimen" prior to running **cobas**® MAI.

Processing of sputum and BAL sediments

- Confirm that the NALC-NaOH-treated sputum and BAL sediment container is properly labeled and contains a minimum of 0.2 mL of specimen. If stored frozen, thaw and equilibrate sample to ambient temperature.
- Vortex sediment sample for a minimum of 10 seconds.
- Transfer a minimum of 0.2 mL and no more than 0.6 mL of sediment specimen into a barcode labeled 5 mL polypropylene screw-cap tube 75x13mm, round base (Sarstedt - Tube P/N 60.504.010, Cap P/N 65.163).

  Note: Prior to specimen transfer confirm that barcode information on the specimen container and the 5 mL secondary tube match.

- Invert the MIS bottles two to four times before use.
- Add five parts of MIS to one part of specimen (e.g., 1 mL of MIS to 0.2 mL of specimen). Close the tube tightly.

  Note: Refer to Table 15.

- Close the MIS bottles immediately after use.
- Shake vigorously 10 to 20 times or vortex for 30-60 seconds.

  Note: One back-and-forth movement is a single shake.

  Note: Ensure that the entire specimen is mixed with MIS.

- Incubate specimen for at least 60 minutes at 15-30°C (room temperature).

  Note: Refer to the "Inactivated specimen storage" section for maximal storage conditions.

- Shake vigorously or vortex for 30-60 seconds.
- Sonicate inactivated specimen according to the "Sonication of specimens" section prior to running **cobas**® MAI.

Table 15 cobas® Microbial Inactivation Solution-treated specimen volume requirements for running **cobas**® MAI

Number of tests to perform from secondary tube	Minimal volume of MIS-treated specimen required	Maximal volume of MIS-treated specimen allowed
1 test order	1.2 mL	3.6 mL
2 test orders*	2.4 mL	3.6 mL
3 test orders*	3.6 mL	3.6 mL

* May be used for processing in mixed-batch with other **cobas**® 6800/8800 assays using the same specimen type or for repeat testing.

Sonication of specimens

- Sonication of specimens for running **cobas**® MAI must be performed using the tube sonicator TS 5 device from Rinco Ultrasonics AG (P/N 46690). The use of other sonication devices may lead to false positive, false negative and/or invalid results. The operation of the instrument is described in detail in the manufacturer's User Guide.
- Place five barcode-labeled closed screw-cap tubes containing 1.2 mL to 3.6 mL of MIS-treated specimen into an MPA rack.

  Note: Ensure that thermostable barcode labels on sample tubes are oriented towards and visible through the slits open at the top on the side of MPA sample racks (see Figure 1).

  Note: Ensure that each tube contains one barcode label.

  Note: Ensure that all five tube positions of the MPA rack are occupied. If less than five tubes containing MIS-treated specimen are available, the remaining positions must be occupied with water-filled or MIS-filled "dummy" tubes of the same tube type and with a barcode label.

Figure 1 Correct placement of sample tubes in MPA rack prior to sonication

- Start the tube sonicator.
- Select the predefined sonication profile "Respiratory Samples."
- Open the tube sonicator device and insert the MPA rack according to the manufacturer's instructions.
- Close the tube sonicator.
- Start the sonication run.
- Confirm that the sonication run was successful and remove the MPA rack.

  Note: Sample tubes are expected to warm up during the sonication run. Exercise caution when removing the MPA rack with sample tubes.

  Note: In case of a sonication failure, refer to the manufacturer's instructions, correct the cause and repeat the sonication run after allowing the samples to cool down for at least 15 minutes.
- MIS-treated and sonicated specimens may now be run with **cobas**® MAI or may be stored according the "Inactivated specimen storage" section.

Running cobas® MAI

cobas® MAI can be run with a minimum required sample volume of 1.2 mL. The operation of the instrument is described in detail in the **cobas**® 6800/8800 Systems User Guide or User Assistance. Figure 2 below summarizes the procedure.

Prior to uncapping tubes and loading specimens onto the **cobas**® 6800/8800 Systems, it is recommended to pellet cell and matrix debris by specimen centrifugation for a maximum of 1 minute at a maximum RCF of 3000 x g.

Note: Vortex specimens for a minimum of 10 seconds if specimens have been stored for more than 1 hour after sonication and before centrifugation.

Note: The omission of the centrifugation step may result in an increased rate of sample clots on the **cobas**® 6800/8800 Systems.

Figure 2 cobas® MAI procedure

1	Log onto the system Press Start to Prepare the system Order Tests • Choose “Raw sputum” for ordering MIS-treated raw sputum specimens • Choose “Sediment” for ordering MIS-treated sputum/BAL sediment specimens
2	Refill reagents and consumables as prompted by the system • Load test specific reagent cassette • Load control cassettes • Load Pipette Tips • Load Processing Plates • Load MGP Reagent • Load Amplification Plates • Refill Specimen Diluent • Refill Lysis Reagent • Refill Wash Reagent
3	Loading specimens onto the system • For each specimen o Uncap tube o Transfer tube to rack • Load sample rack and clot tip racks into the sample supply module • Confirm samples have been accepted into the transfer module
4	Start run
5	Review and export results
6	Remove and cap any sample tubes meeting the minimum volume requirements if needed for future use Clean up instrument • Unload empty control cassettes • Empty amplification plate drawer • Empty liquid waste • Empty solid waste

Results

cobas® MAI automatically detects and differentiates *M. avium* and *M. intracellulare* DNA for samples and controls, displaying test validity, as well as individual target results.

Quality control and validity of results

- One **cobas**® Buffer Negative Control [(-) Ctrl] and one MAI Positive Control [MAI (+) C] are processed with each batch of a requested result type.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure batch validity.
- All flags are described in the **cobas**® 6800/8800 Systems User Guide or User Assistance.
- The batch is valid if no flags appear for all controls. If the batch is invalid, repeat testing of the entire batch.

Validation of batch results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control performance, and validation of individual sample results is performed by the **cobas**® 6800/8800 software based on internal control results.

Interpretation of results

Figure 3 Example of **cobas**® MAI results

Test	Sample ID	Valid	Flags	Sample type	Overall result	Target 1	Target 2
MAI 850 ul	MAI_R_0001	NA		Raw sputum	**NA**	MIN Negative	MAV Positive
MAI 850 ul	MAI_R_0002	NA		Raw sputum	**NA**	MIN Positive	MAV Negative
MAI 850 ul	MAI_R_0003	NA	P02T	Raw sputum	**NA**	Invalid	Invalid
MAI 850 ul	MAI_S_0001	NA		Sediment	**NA**	MIN Negative	MAV Positive
MAI 850 ul	MAI_S_0002	NA		Sediment	**NA**	MIN Positive	MAV Negative
MAI 850 ul	MAI_S_0003	NA	C02H1	Sediment	**NA**	Invalid	Invalid
MAI 850 ul	C161420284090428828404	Yes		(-) Ctrl	**Valid**	Valid	Valid
MAI 850 ul	C161420284093009580264	Yes		MAI (+) C	**Valid**	Valid	Valid

Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid sample results.
- The "Valid" and "Overall Result" columns are not applicable (NA) to sample results for the **cobas**® MAI and are marked with "NA". Values reported in these columns are not applicable and **do not** impact the validity of results reported within individual Target Result columns.
- Reported target results for individual samples are valid unless indicated as "Invalid" within the individual target result column.
- Results of this test should only be interpreted in conjunction with information available from clinical evaluation of the patient and patient history.

Results and their corresponding interpretation for detecting MAI are shown in Table 16.

Table 16 cobas® MAI results and interpretation

Target 1	Target 2	Interpretation
MIN Positive	**MAV Positive**	All requested results were valid. Target signal detected for *M. intracellulare* and *M. avium* DNA.
MIN Positive	**MAV Negative**	All requested results were valid. Target signal detected for *M. intracellulare* DNA. No target signal detected for *M. avium* DNA.
MIN Positive	**Invalid**	Not all requested results were valid. Target signal detected for *M. intracellulare* DNA. *M. intracellulare* result is valid. *M. avium* result is invalid. Original specimen should be re-tested to obtain valid *M. avium* results. If the result is still invalid, a new specimen should be obtained.
MIN Negative	**MAV Positive**	All requested results were valid. No target signal detected for *M. intracellulare* DNA. Target signal detected for *M. avium* DNA.
MIN Negative	**MAV Negative**	All requested results were valid. No target signal detected for *M. intracellulare* DNA. No target signal detected for *M. avium* DNA.
MIN Negative	**Invalid**	Not all requested results were valid. No target signal detected for *M. intracellulare* DNA. *M. intracellulare* result is valid. *M. avium* result is invalid. Original specimen should be re-tested to obtain valid *M. avium* results. If the result is still invalid, a new specimen should be obtained.
Invalid	**MAV Positive**	Not all requested results were valid. *M. intracellulare* result is invalid. Original specimen should be re-tested to obtain valid *M. intracellulare* results. If the result is still invalid, a new specimen should be obtained. Target signal detected for *M. avium* DNA. *M. avium* result is valid.
Invalid	**MAV Negative**	Not all requested results were valid. *M. intracellulare* result is invalid. Original specimen should be re-tested to obtain valid *M. intracellulare* results. If the result is still invalid, a new specimen should be obtained. No target signal detected for *M. avium* DNA. *M. avium* result is valid.
Invalid	**Invalid**	Both *M. intracellulare* and *M. aviuim* results are invalid. Original specimen should be re-tested to obtain valid *M. intracellulare* and *M. avium* results. If the results are still invalid, a new specimen should be obtained.

Procedural limitations

- **cobas**® MAI should always be performed along with culture to minimize the risk of false negative results, as well as to allow for drug susceptibility testing of the MAC isolate to aid in patient management.
- The performance of **cobas**® MAI has been validated for raw sputum and for sputum and BAL sediment specimens that have been liquefied, decontaminated and concentrated using NALC-NaOH. The use of other sample types may lead to false positive, false negative and/or invalid results.
- Digestion and decontamination should be performed using NALC-NaOH procedures recommended by the CDC.[19] The use of alternative pre-analytic sample preparation procedures may lead to false positive, false negative and/or invalid results.
- **cobas**® MAI has been validated for use with raw sputum and NALC-NaOH-treated sputum and BAL sediment specimens chemically inactivated using MIS. Other inactivation procedures have not been evaluated and may lead to false positive, false negative and/or invalid results.
- Success in mycobacterial inactivation depends on adherence to procedures outlined in this document and complete mixing of sample with MIS. Pre-analytic treatment of patient samples by MIS reduces, but may not completely eliminate the risk of mycobacterial infection.
- Exceeding volume limitations and/or deviating from the procedural steps outlined in "Processing of raw sputum specimens", "Processing of sputum and BAL sediments" and "Sonication of specimens" sections may lead to false positive, false negative and/or invalid results.
- Nucleic Acid Amplification assays are unable to determine viability of organism.
- Therapeutic success or failure cannot be determined using this test.
- Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 6800/8800 Systems.
- **cobas**® MAI has been evaluated only for use in combination with the **cobas**® MAI Positive Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems, the MIS, and the tube sonicator TS 5 from Rinco Ultrasonics AG.
- Reliable results depend on proper sample collection, storage, and handling procedures.
- **cobas**® MAI has not been evaluated in patients younger than 18 years of age.
- **cobas**® MAI is not indicated for use with respiratory specimens from patients being treated with anti-mycobacterial drug therapy, for monitoring treatment response or as a test for cure.
- **cobas**® MAI distinguishes between the *M. intracellulare* and *M. avium*. Other species of the *M. avium* complex are detected by **cobas**® MAI but are not differentiated. They are either detected in the *M. intracellulare* or the *M. avium* target. Refer to the inclusivity study within the "Performance Evaluation" section for details.
- Detection of *M. avium complex* is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, and patient factors (i.e., age, severity of disease, HIV status).
- For patients who are both MAC and HIV infected, there is a higher likelihood of specimens being smear microscopy negative and therefore having MAC DNA present at levels below the assay's limit of detection.
- Health care providers must interpret results in the context of the patient's history, clinical presentation, as well as other laboratory and radiography test results.

- False negative or invalid results may occur due to polymerase inhibition. The Internal Control is included in **cobas**® MAI to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.
- The addition of AmpErase enzyme into the **cobas**® MAI Master Mix reagent enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents.
- Though rare, mutations within the highly conserved regions of the genomic DNA of *M. avium* complex covered by **cobas**® MAI primers and/or probes may result in failure to detect the presence of the bacterium.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to another, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies.
- Use of tubes other than those recommended in Table 11 must be verified by user prior to implementation into **cobas**® MAI workflow in the laboratory. Use of other tube types may result in damage to the tubes and contamination of sonicator surfaces. False negative results due to insufficient sonication energy transfer may also occur.
- Use of barcodes other than those recommended in Table 11 must be verified by user prior to implementation into **cobas**® MAI workflow in the laboratory. Use of other barcodes may result in damage to the barcode.

Performance evaluation

Key performance characteristics

Sample inactivation

The reduction of mycobacterial infection risk by treating samples with MIS was evaluated using high positive cultures of two MTB complex strains (MTB CDC268 and MTB H37) at three different sites and using three different MIS reagent lots. For each condition five culture aliquots of concentration levels up to 5 x 10^7 CFU/mL were treated with MIS in a 1:2 ratio for 60 minutes at room temperature. The samples were then centrifuged for 15 minutes at 3000 x g, washed twice with sterile PBS and finally resuspended in 0.5mL of sterile PBS. At two sites, the entire inactivated sample was inoculated and tested for growth using the BACTEC™ MGIT™ 320 Mycobacterial Detection System (Becton Dickinson). At the third site, MTB viability was tested on solid Löwenstein-Jensen (LJ) medium. None of the inactivated samples showed growth of *M. tuberculosis* complex bacteria at the end of the 56-day incubation period.

Limit of Detection (LoD)

The limit of detection of **cobas**® MAI was determined by analysis of serial dilutions of one *M. intracellulare* strain (ATCC® 13209™) and one *M. avium* strain (ATCC® 19075™) each, in two pooled negative clinical matrices - raw sputum and sputum/BAL sediments. Panels of concentration levels plus a blank were tested by a total of 72 replicates per concentration using three lots of **cobas**® MAI test reagents over multiple runs, days, operators, and instruments.

The LoD for *M. intracellulare* ranged from 46.3 CFU/mL (sputum/BAL sediment) to 46.6 CFU/mL (raw sputum).

The LoD for *M. avium* ranged from 43.5 CFU/mL (sputum/BAL sediment) to 44.9 CFU/mL (raw sputum).

Inclusivity

The inclusivity of **cobas**® MAI for eleven members of the *M. avium* complex was confirmed by testing a total of 25 strains.

The following species were detected and generated *M. intracellulare* positive results:

- *M. intracellulare* (ATCC® 25130™, ATCC® 35763™, B99-03.25.0163, B99-04.23.0178, B00-08.20.1090, B99-05.19.0190, B98-10.30.0156)
- *M. arosiense* (E. Tortoli)
- *M. chimaera* (HO1421839)
- *M. colombiense* (DSM 45105)
- *M. indicus pranii* (DSM 45239)
- *M. marseillense* (CCUG 56325 T)
- *M. timonense (*11324/16*)*
- *M. vulneris* (DMS 45247)
- *M. yongonense* (B04-09.20.0164)

The following species were detected and generated *M. avium* positive results:

- *M. avium* (N-315 and N-337, culture isolate from Japanese patients)
- *M. avium* supsp. a*vium* (B95-X25 serotype 3, B95-25522 serotype 8, B95-18302 serotype 15, ATCC® 35718™)
- *M. avium* supsp. *hominissuis* (ITM 960255)
- *M. avium* supsp. *paratuberculosis* (B98-11.02.0221)
- *M. avium* supsp. *silvaticum* (DSM 44157)
- *M. bouchedurhonense* (CCUG 56331)

All strains were detected at 241 CFU/mL and 256 CFU/mL for *M. intracellulare* and *M. avium*, respectively, using the sediment specimen type.

Precision

In-house precision was examined using a panel composed of *M. intracellulare* (ATCC® 13209™) and *M. avium* (ATCC® 19075™) cultures diluted into two pooled negative clinical matrices - raw sputum and sputum/BAL sediments. Sources of variability were examined with a panel consisting of three concentration levels, using three lots of **cobas**® MAI reagents and two instruments over a time course of 12 days and with a total of 24 runs. A description of the precision panels and the observed positivity rates are shown in Table 17 and Table 18. All negative panel members tested negative throughout the study. Analysis of standard deviation and percent coefficient of variation of the Ct values from tests performed on positive panel members (see Table 19 and Table 20) yielded overall CV (%) ranging from 1.5% to 2.7% for *M. intracellulare* and from 1.5% to 2.5% for *M. avium*.

Table 17 Summary of within laboratory precision – *M. intracellulare*

Target Concentration	N Tested	N MIN Positive	MIN Positivity Rate	95% Confidence Interval	
				Lower Limit	Upper Limit
M. intracellulare - raw sputum					
Negative	48	0	0.0%	0.0%	7.4%
77.4 CFU/mL	48	48	100.0%	92.6%	100.0%
232 CFU/mL	48	48	100.0%	92.6%	100.0%
M. intracellulare – sediment					
Negative	48	0	0.0%	0.0%	7.4%
74.3 CFU/mL	48	48	100.0%	92.6%	100.0%
223 CFU/mL	48	48	100.0%	92.6%	100.0%

Table 18 Summary of within laboratory precision – *M. avium*

Target Concentration	N Tested	N MAV Positive	MAV Positivity Rate	95% Confidence Interval	
				Lower Limit	Upper Limit
M. avium - raw sputum					
Negative	48	0	0.0%	0.0%	7.4%
88.0 CFU/mL	48	48	100.0%	92.6%	100.0%
264 CFU/mL	48	48	100.0%	92.6%	100.0%
M. avium - sediment					
Negative	48	0	0.0%	0.0%	7.4%
71.1 CFU/mL	48	48	100.0%	92.6%	100.0%
213 CFU/mL	48	48	100.0%	92.6%	100.0%

Table 19 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, *M. intracellulare* positive panels

Target Concentration	Positivity Rate	Mean Ct	Within run		Between run		Between day		Between instrument		Between lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
M. intracellulare - raw sputum														
77.4 CFU/mL	100.0%	37.6	0.83	2.2	0.00	0.0	0.48	1.3	0.20	0.5	0.00	0.0	0.98	2.6
232 CFU/mL	100.0%	36.5	0.74	2.0	0.47	1.3	0.33	0.9	0.00	0.0	0.29	0.8	0.98	2.7
M. intracellulare – sediment														
74.3 CFU/mL	100.0%	38.1	0.56	1.5	0.34	0.9	0.00	0.0	0.17	0.4	0.13	1.8	0.69	1.8
223 CFU/mL	100.0%	36.9	0.37	1.0	0.25	0.7	0.00	0.0	0.33	0.9	0.00	0.0	0.56	1.5

Table 20 Overall mean, standard deviations and coefficients of variation (%) for cycle threshold, *M. avium* positive panels

Target Concentration	Positivity Rate	Mean Ct	Within run		Between run		Between day		Between instrument		Between lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
M. avium - raw sputum														
88.0 CFU/mL	100.0%	37.9	0.87	2.3	0.00	0.0	0.25	0.7	0.24	0.6	0.00	0.0	0.94	2.5
264 CFU/mL	100.0%	36.4	0.48	1.3	0.40	1.1	0.35	1.0	0.16	0.4	0.00	0.0	0.73	2.0
M. avium – sediment														
71.1 CFU/mL	100.0%	38.8	0.51	1.3	0.25	0.7	0.10	0.3	0.00	0.0	0.11	0.3	0.59	1.5
213 CFU/mL	100.0%	37.5	0.50	1.3	0.34	0.9	0.40	1.0	0.00	0.0	0.12	0.3	0.74	2.0

Analytical specificity/cross reactivity

A panel of 173 bacteria, fungi and viruses, including those commonly found in respiratory tract, were tested with **cobas®** MAI to assess analytical specificity. The organisms listed in Table 21 were tested at concentrations of approximately 1 x 10^6 units/mL for bacteria and approximately 1 x 10^5 units/mL for viruses. Testing was performed with each potential interfering organism in absence and presence of *M. intracellulare*/*M. avium* target (at 200 CFU/mL). None of the organisms interfered with the test performance by generating false positive results. Detection of *M. intracellulare*/*M. avium* target was not affected by organisms tested except *M. kansasii* and *M. szulgai* at concentration levels > 1E+05 CFU/mL and *M. gastri* at concentration levels > 1E+04 CFU/mL.

Potential cross-reactivity of *Histoplasma capsulatum*, *Mycobacterium africanum, Mycobaterium leprae*, *Mycobacterium microti, Mycobacterium pinnipedii* and *Mycobacterium suricattae* was evaluated *in silico*. The results of the *in silico* analyses predict a low likelihood of amplification and detection of those organisms when using **cobas®** MAI.

Table 21 Microorganisms tested for analytical specificity/cross reactivity

Microorganism	Concentration	Microorganism	Concentration
Acinetobacter baumannii	1.0E+06 CFU/mL	*Mycobacterium gordonae*	1.0E+06 CFU/mL
Acinetobacter calcoaceticus	1.0E+06 CFU/mL	*Mycobacterium haemophilum*	1.0E+06 CFU/mL
Actinomyces israelii	1.0E+06 CFU/mL	*Mycobacterium holsaticum*	1.0E+06 CFU/mL
Actinomyces odontolyticus	1.0E+06 CFU/mL	*Mycobacterium intermedium*	1.0E+06 CFU/mL
Adenovirus	1.0E+05 U/mL	*Mycobacterium kansasii*	1.0E+05 CFU/mL*
Aeromonas hydrophila	1.0E+06 CFU/mL	*Mycobacterium kumamontonense*	1.0E+06 CFU/mL
Aspergillus fumigatus	1.0E+06 CFU/mL	*Mycobacterium lentiflavum*	1.0E+06 CFU/mL
Bacillus cereus	1.0E+06 CFU/mL	*Mycobacterium malmoense*	1.0E+06 CFU/mL
Bacillus subtilis subsp. *subtilis*	1.0E+06 CFU/mL	*Mycobacterium mantenii*	1.0E+06 CFU/mL
Bactericides fragilis	1.0E+06 CFU/mL	*Mycobacterium marinum*	1.0E+06 CFU/mL
Blastomyces dermatitidis	1.0E+06 geq/mL	*Mycobacterium mucogenicum*	1.0E+06 CFU/mL
Bordetella parapertussis	1.0E+06 CFU/mL	*Mycobacterium neoaurum*	1.0E+06 CFU/mL
Bordetella pertussis	1.0E+06 CFU/mL	*Mycobacterium nonchromogeicum*	1.0E+06 CFU/mL
Burkholderia cepacia	1.0E+06 CFU/mL	*Mycobacterium orygis*	1.0E+06 CFU/mL
Campylobacter jejuni subsp. *jejuni*	1.0E+06 CFU/mL	*Mycobacterium peregrinum*	1.0E+06 CFU/mL
Candida albicans	1.0E+06 CFU/mL	*Mycobacterium scrofulaceum*	1.0E+06 CFU/mL
Candida glabrata	1.0E+06 CFU/mL	*Mycobacterium simiae*	1.0E+06 CFU/mL
Candida krusei	1.0E+06 CFU/mL	*Mycobacterium smegmatis*	1.0E+06 CFU/mL
Candida parapsilosis	1.0E+06 CFU/mL	*Mycobacterium szulgai*	1.0E+05 CFU/mL*
Candida tropicalis	1.0E+06 CFU/mL	*Mycobacterium terrae*	1.0E+06 CFU/mL
Chlamydia trachomatis	1.0E+06 IFU/mL	*Mycobacterium thermoresistible*	1.0E+06 CFU/mL
Chlamydophila pneumoniaea	1.0E+06 IFU/mL	*Mycobacterium triviale*	1.0E+06 CFU/mL
Chromobacterium violaceum	1.0E+06 CFU/mL	*Mycobacterium tuberculosis*	1.0E+06 CFU/mL
Citrobacter freundii	1.0E+06 CFU/mL	*Mycobacterium vaccae*	1.0E+06 CFU/mL
Clostridium perfringens	1.0E+06 CFU/mL	*Mycobacterium xenopi*	1.0E+06 CFU/mL
Corynebacterium diphtheriae	1.0E+06 CFU/mL	*Mycoplasma pneumoniae*	1.0E+06 ccu/mL
Corynebacterium jeikeium	1.0E+06 CFU/mL	*Neisseria gonorrhoeae*	1.0E+06 CFU/mL
Corynebacterium pseudodiptheriticum	1.0E+06 CFU/mL	*Neisseria lactamica*	1.0E+06 CFU/mL
Corynebacterium ulcerans	1.0E+06 geq/mL	*Neisseria meningitides*	1.0E+06 CFU/mL
Corynebacterium xerosis	1.0E+06 CFU/mL	*Neisseria mucosa*	1.0E+06 CFU/mL
Cryptococcus neoformans	1.0E+06 CFU/mL	*Neisseria sicca*	1.0E+06 CFU/mL
Cytomegalovirus	1.0E+05 IFU/mL	*Nocardia asteroides*	1.0E+06 CFU/mL
Eikenella corrodens	1.0E+06 CFU/mL	*Nocardia brasiliensis*	1.0E+06 geq/mL
Enterobacter aerogenes	1.0E+06 CFU/mL	*Nocardia cyriacigeorgica*	1.0E+06 CFU/mL
Enterobacter cloacae subsp. *cloacae*	1.0E+06 CFU/mL	*Nocardia farcinica*	1.0E+06 CFU/mL
Enterococcus avium	1.0E+06 CFU/mL	*Nocardia nova*	1.0E+06 CFU/mL
Enterococcus faecalis	1.0E+06 CFU/mL	*Nocardia otitidiscaviarum*	1.0E+06 CFU/mL
Enterococcus faecium	1.0E+06 CFU/mL	*Nocardia transvalensis*	1.0E+06 CFU/mL
Enterovirus Type 68 / 2007	1.0E+05 U/mL	*Pasteurella multocida* subsp. *tigris*	1.0E+06 CFU/mL

Microorganism	Concentration	Microorganism	Concentration
Escherichia coli	1.0E+06 CFU/mL	*Pediococcus acidilactici*	1.0E+06 geq/mL
Escherichia coli producing CTX-M-15 ESBL	1.0E+06 CFU/mL	*Pediococcus pentosaceus*	1.0E+06 CFU/mL
Fusobacterium nucleatum subsp. *nucleatum*	1.0E+06 CFU/mL	*Penicillium chermesinum*	1.0E+06 CFU/mL
Gordona rubropertinctus	1.0E+06 geq/mL	*Peptostreptococcus anaerobius*	1.0E+06 CFU/mL
Haemophilus influenzae	1.0E+06 CFU/mL	*Peptostreptococcus magnus*	1.0E+06 CFU/mL
Haemophilus parahaemolyticus	1.0E+06 CFU/mL	*Porphyromonas asaccharolytica*	1.0E+06 CFU/mL
Haemophilus parainfluenzae	1.0E+06 CFU/mL	*Prevotella melaninogenica*	1.0E+06 CFU/mL
Herpes simplex virus Type 1	1.0E+05 cp/mL	*Propionibacterium acnes*	1.0E+06 CFU/mL
Herpes simplex virus Type 2	1.0E+05 cp/mL	*Proteus mirabilis*	1.0E+06 CFU/mL
Human Immunodeficiency Virus	1.0E+05 cp/mL	*Proteus vulgaris*	1.0E+06 CFU/mL
Human influenza virus A	1.0E+05 U/mL	*Providencia stuartii*	1.0E+06 CFU/mL
Human influenza virus B	1.0E+05 U/mL	*Pseudomonas aeruginosa*	1.0E+06 CFU/mL
Human metapneumovirus	1.0E+05 U/mL	*Rhizopus* spp.	1.0E+06 CFU/mL
Human parainfluenza virus type 1	1.0E+05 U/mL	*Rhodococcus equi*	1.0E+06 CFU/mL
Human parainfluenza virus type 2	1.0E+05 U/mL	Rubella virus	1.0E+05 U/mL
Human parainfluenza virus type 3	1.0E+05 U/mL	Rubeola virus	1.0E+05 U/mL
Human parainfluenza virus type 4	1.0E+05 U/mL	Rubula virus	1.0E+05 U/mL
Human respiratory syncytial virus A	1.0E+05 U/mL	*Salmonella enterica* subsp. *enterica* serovar Dublin	1.0E+06 CFU/mL
Human respiratory syncytial virus B	1.0E+05 U/mL	*Scedosporium* spp.	1.0E+06 CFU/mL
Human rhinovirus 16	1.0E+05 U/mL	*Serratia marcescens* subsp. *marcescens*	1.0E+06 CFU/mL
Kingella kingae	1.0E+06 CFU/mL	*Shigella flexneri*	1.0E+06 CFU/mL
Kingella oralis	1.0E+06 CFU/mL	*Shigella sonnei*	1.0E+06 CFU/mL
Klebsiella oxytoca	1.0E+06 CFU/mL	*Staphylococcus aureus* subsp. *aureus*	1.0E+06 CFU/mL
Klebsiella pneumoniae producing KPC-3	1.0E+06 CFU/mL	*Staphylococcus capitis* subsp. *capitis*	1.0E+06 CFU/mL
Klebsiella pneumoniae subsp. *pneumoniae*	1.0E+06 CFU/mL	*Staphylococcus epidermidis*	1.0E+06 CFU/mL
Lactobacillus acidophilus	1.0E+06 CFU/mL	*Staphylococcus haemolyticus*	1.0E+06 CFU/mL
Lactobacillus casei	1.0E+06 CFU/mL	*Staphylococcus hominis* subsp. *hominis*	1.0E+06 CFU/mL
Legionella micdadei	1.0E+06 CFU/mL	*Staphylococcus lugdunensis*	1.0E+06 CFU/mL
Legionella pneumophila subsp.*pneumophila*	1.0E+06 CFU/mL	*Stenotrophomonas maltophilia*	1.0E+06 CFU/mL
Leuconostoc mesenteroides subsp. *mesenteroides*	1.0E+06 CFU/mL	*Streptococcus agalactiae*	1.0E+06 CFU/mL
Listeria monocytogenes	1.0E+06 CFU/mL	*Streptococcus constellatus* subsp. *constellatus*	1.0E+06 CFU/mL
Moraxella catarrhalis	1.0E+06 CFU/mL	*Streptococcus equi* subsp. *equi*	1.0E+06 CFU/mL
Morganella morganii subsp. *morganii*	1.0E+06 CFU/mL	*Streptococcus mitis*	1.0E+06 CFU/mL
Mycobacterium abscessus	1.0E+06 CFU/mL	*Streptococcus mutans*	1.0E+06 CFU/mL
Mycobacterium asiaticum	1.0E+06 CFU/mL	*Streptococcus parasanguinis*	1.0E+06 CFU/mL
Mycobacterium bovis BCG	1.0E+06 CFU/mL	*Streptococcus pneumoniae*	1.0E+06 CFU/mL
Mycobacterium bovis subsp. *bovis*	1.0E+06 CFU/mL	*Streptococcus pyogenes*	1.0E+06 CFU/mL
Mycobacterium bovis subsp. *caprae*	1.0E+06 CFU/mL	*Streptococcus salivarius* subsp. *salivarius*	1.0E+06 CFU/mL
Mycobacterium canetti	1.0E+06 CFU/mL	*Streptococcus sanguinis*	1.0E+06 CFU/mL
Mycobacterium caprae	1.0E+06 CFU/mL	*Streptococcus uberis*	1.0E+06 CFU/mL
Mycobacterium celatum	1.0E+06 CFU/mL	*Streptomyces anulatus*	1.0E+06 CFU/mL
Mycobacterium chelonae	1.0E+06 CFU/mL	*Streptomyces griseinus*	1.0E+06 CFU/mL
Mycobacterium chubuense	1.0E+06 CFU/mL	*Tsukamurella* spp.	1.0E+06 geq/mL
Mycobacterium confluentis	1.0E+06 CFU/mL	Varicella Zoster Virus	1.0E+05 cp/mL

Microorganism	Concentration	Microorganism	Concentration
Mycobacterium flavescens	1.0E+06 CFU/mL	*Veillonella atypica*	1.0E+06 CFU/mL
Mycobacterium fortuitum	1.0E+06 CFU/mL	*Veillonella parvula*	1.0E+06 CFU/mL
Mycobacterium fuerth	1.0E+06 CFU/mL	*Weissella paramesenteroides*	1.0E+06 CFU/mL
Mycobacterium gastri	1.0E+04 CFU/mL*	-	-

* Level at which no interference with *M. intracellulare* and *M. avium* detection observed, tested also at 1.0E+06 CFU/mL which showed interference with both, *M. intracellulare* and *M. avium* targets.

Interference

The effect of exogenous substances potentially secreted into respiratory specimens was evaluated (Table 22). Each potentially interfering substance was tested at or above clinically relevant levels in contrived sputum specimens in absence and presence of *M. intracellulare* and *M. avium* target (co-spiked at 200 CFU/mL).

None of the substances interfered with the test performance by generating false-negative or false-positive results.

Table 22 List of exogenous substances tested for interference

Substance	Concentration	Substance	Concentration
Albuterol sulfate	0.5 µg/mL	Kanamycin monosulfate	240 µg/mL
Amikacin	80.1 µg/mL	Levofloxacin	5 mg/mL
Amoxicillin	86.4 µg/mL	Lidocaine HCl	1.2 % (w/v)
Beclomethasone	3459 pg/mL	Menthol	0.50% (w/v)
Benzocaine	1.2% (w/v)	Methyl salicylate	0.06% (v/v)
Budesonide	3 mg/mL	Mometasone	100 µg/mL
Butterbur	225 mg/mL	Moxifloxacin	15 µg/mL
Capreomycin	80 µg/mL	Mupirocin	5% (w/v)
Cetylpyridinium chloride	0.5% (w/v)	NaCl	5% (w/v)
Chlorhexidine gluconate	1% (v/v)	Nicotine	1 µg/mL
Cicloserin (Cycloserine)	105 µg/mL	Nystatin	1% (v/v)
Clarithromycin	20 µg/mL	Oxymetazoline	12 ng/mL
Dexamethasone	601 ng/mL	Pentamidine	1366 ng/mL
Ephedrine hydrochloride	1 mg/mL	Phenylephrine	5 mg/mL
Epinephrine	100 pg/mL	Prednisolone	3 µg/mL
Ethambutol	50 µg/mL	Pyrazinamide	240 µg/mL
Ethionamide	15 µg/mL	Rifampicin	25 µg/mL
Eucalyptol	0.002% (v/v)	Stinging Nettle Extract (500 mg)	5 mg
Flunisolide	400 µg/mL	Streptomycin	240 µg/mL
Fluticasone Propionate	5 µg/ml	Sulfur	0.01% (w/v)
Formoterol Fumarate Dihydrate	66 µg/mL	Tea Tree Oil	0.50% (v/v)
Goldenseal root (capsules 570 mg)	5.7 mg	Theophylline	20 µg/mL
Guaifenesin	5 mg/mL	Tobramycin	24.1 µg/mL
Isoniazid	50 µg/mL	Zanamivir	10 mg/mL

Endogenous substances that may be present in respiratory specimens were tested for interference (Table 23). Each potentially interfering substance was tested at or above clinically relevant levels in contrived sputum specimens in absence and presence of *M. intracellulare* and *M. avium* target (co-spiked at 200 CFU/mL).

None of the substances interfered with the test performance by generating false-positive results. None of the substances except 5% mucin interfered with the test performance by generating false-negative results. No interference was observed for mucin at concentration levels at or below 4%.

Table 23 List of endogenous substances tested for interference

Substance	Concentration	Substance	Concentration
Gastric juice	10% (v/v)	Mucin	4%*
Hemoglobin	2 g/L	Pus	5%
Human Whole Blood	5 % (v/v)	Saliva	10% (v/v)
hDNA	4 mg/L	-	-

* Level at which no interference with *M. intracellulare* and *M. avium* detection observed, tested also at 5% which showed partial interference with both, *M. intracellulare* and *M. avium* targets.

Whole system failure

The samples tested in the whole system failure study were contrived sputum and sputum sediment specimens co-spiked with *M. intracellulare* and *M. avium* target to a concentration of approximately 3 x LoD of the respective matrix. The results of this study determined that all replicates were valid and positive for *M. intracellulare* and *M. avium*, resulting in a whole system failure rate of 0% with an upper one-sided 95% confidence interval of 3.0%.

Cross contamination

Potential cross contamination on the **cobas**® 6800/8800 Systems using **cobas**® MAI has been studied using the related **cobas**® MTB test with identical sample types and workflows. Cross contamination can cause false positive results. In this performance study the sample to sample cross contamination rate has been determined to be 0.0% (0/240) when alternating very high positive and negative samples were tested over multiple runs. Testing was done using contrived sputum sediment samples spiked with MTB complex target at 2 x 10^6 CFU/mL, a sample concentration generating Ct values earlier than in 95% of specimens from the infected patients in the intended use population.

Performance using clinical specimens

The performance of **cobas**® MAI using clinical samples was evaluated by testing prospective and archived specimens (raw sputum, sputum/BAL sediments) from subjects with presumptive mycobacterial respiratory infection collected in Germany, Japan, South Africa, Switzerland and Texas. Side-by-side comparison testing with the COBAS® TaqMan® MAI Test was performed. Sensitivity and specificity were established in comparison to culture. The patient population for sensitivity comprised 51 AFB smear negative (49%), 13 AFB smear scanty (13%), 19 AFB smear 1+ (18%), 15 AFB smear 2+ (14%), 4 AFB smear 3+ (4%) and 2 AFB smear indeterminate (2%) for sputum/BAL sediments with a total of 81 sputum sediments and 23 BAL sediments. For raw sputum 26 AFB smear negative (47%), 5 AFB smear scanty (9%), 8 AFB smear 1+ (15%), 12 AFB smear 2+ (22%) and 4 AFB smear 3+ (7%) were tested.

Results are shown in Table 24.

Table 24 Sensitivity and specificity of **cobas®** MAI using clinical samples

				Roche **cobas® MAI**	Roche **COBAS® TaqMan® MAI**
Sensitivity	**Raw Sputum**	**MIN C+**	MIN	16/24 **66.7%** [44.7-84.4%]	N/A
		MAV C+	MAV	27/31 **87.1%** [70.1- 96.3%]	N/A
		MIN a/o MAV C+	MIN/MAV	44/55 **80.0%** [67.0 – 89.6%]	N/A
	Sediment	**MIN C+**	MIN	27/46 **58.7%** [43.2-73.0%]	32/46 **69.6%** [54.2-82.3%]
		MAV C+	MAV	35/58 **60.3%** [46.6- 72.9%]	36/58 **62.1%** [48.4 – 74.5%]
		MIN a/o MAV C+	MIN/MAV	62/104 **59.6%** [49.5 – 69.1%]	68/104 **65.4%** [55.4 – 74.4%]
Specificity	**Raw Sputum**	**MIN C-**	MIN	350/350 **100.0%** [99.0 – 100.0%]	N/A
		MAV C-	MAV	350/350 **100.0%** [99.0 – 100.0%]	N/A
		MIN and MAV C-	MIN/MAV	350/350 **100.0%** [99.0 – 100.0%]	N/A
	Sediment	**MIN C-**	MIN	412/412 **100.0%** [99.1 – 100.0%]	408/412 **99.0%** [97.5 – 99.7%]
		MAV C-	MAV	412/412 **100.0%** [99.1 – 100.0%]	411/412 **99.8%** [98.7 – 100.0%]
		MIN and MAV C-	MIN/MAV	412/412 **100.0%** [99.1 – 100.0%]	407/412 **98.8%** [97.2 – 99.6%]

C = Culture

Additional information

Key assay features

Sample types	• Raw sputum
	• NALC-NaOH-treated sputum and BAL sediments
Amount of sample processed	• ≥ 0.4 mL of patient sample treated with MIS in ratio 1:2 (total volume ≥ 1.2 mL) required in sample tube for raw sputum, instrument processes 0.85 mL
	• ≥ 0.2 mL of patient sample treated with MIS in ratio 1:5 (total volume ≥ 1.2 mL) required in sample tube for sputum/BAL sediment, instrument processes 0.85 mL
Test duration	• < 3.5 hours to first result

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 25 Symbols used in labeling for Roche PCR diagnostics products

	Ancillary Software		*In Vitro* diagnostic medical device
	Authorized representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD performance evaluation only		Global Trade Item Number
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		

 This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 26 Manufacturer and distributors

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
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Roche Diagnostics GmbH
Sandhofer Strasse 116
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Roche Diagnostics, SL
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Roche Diagnostica Brasil Ltda.
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Roche Diagnostics
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Pour toute assistance technique,
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Distribuidor em Portugal:
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Document revision

Document Revision Information	
Doc Rev. 1.0 06/2018	First Publishing.
Doc Rev. 2.0 04/2019	Clarified sample types in the **Intended use** and **Explanation of the test** sections. Updated and clarified Open-kit stablility for **cobas**® MAI. Added additional Solid Waste Bag option. Corrected the LoD for *M. intracellulare* in sputum/BAL sediment. Corrected the Precision target concentration for *M. avium* - raw sputum. Please contact your local Roche Representative if you have any questions.