Rx Only

cobas® MAI

Nucleic acid test
for use on the cobas® 5800/6800/8800 Systems

For in vitro diagnostic use

cobas® MAI	P/N: 09040595190
For use on the cobas® 5800 System	
cobas® MAI Positive Control Kit	P/N: 09040609190
cobas® Buffer Negative Control Kit	P/N: 09051953190
For use on the cobas® 6800/8800 Systems	
cobas® MAI Positive Control Kit	P/N: 07544863190 or P/N: 09040609190
cobas® Buffer Negative Control Kit	P/N: 07002238190 or P/N: 09051953190

Table of contents

Intended use

cobas® MAI for use on the **cobas**® 5800/6800/8800 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 mycobacterial culture as an aid in the diagnosis of pulmonary *M. avium* and *M. intracellulare* infections.

Summary and explanation of the test

Background

M. avium and *M. intracellulare* are two closely related but distinct species of slowly growing nontuberculous mycobacteria (NTM), that are member of the *M. avium* complex (MAC). 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; pulmonary infections (MAC, *M. kansasii* and *M. abscessus*), lymphonodal infections, seen commonly in pediatric populations, (MAC, *M. scrofulaceum*, *M. malmoense*), disseminated disease in severely immunocompromised patients, and skin and soft tissue or bone and joint 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] *M. avium* and *M. intracellulare* (MAI) are the two members of the MAC that are most commonly associated with human disease.[5]

MAI are primarily pulmonary pathogens that often affect individuals who are immune compromised (e.g., patients with AIDS, hairy cell leukemia, or on immunosuppressive chemotherapy). MAI are 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 MAI infections are associated with chronic lung diseases, such as COPD, chronic bronchitis, bronchiectasis, cystic fibrosis, and lung cancer. MAI may also cause osteomyelitis, tenosynovitis, synovitis, and disseminated MAI infection can involve lymph nodes, CNS, liver, spleen and bone marrow. Cutaneous infections occur usually through direct inoculation. MAI 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 MAI infections. *M. intracellulare* is responsible for 40% of infections in immunocompetent patients. MAI lung disease usually shows two types of clinical presentation such as an apical fibrocavitary lung disease or as the Lady Windermere syndrome that is associated with nodular or fibronodualr lung infitlriates and 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 disseminated MAI 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 MAI 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 mycobacterial culture is recommended for diagnosis. Diagnosis requires:

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

(ii) positive mycobacterial 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 for other drugs.[5]

The presumptive diagnosis of MAC infection can be established based on both clinical presentation and radiographic findings, and confirmed by recovery of the organism in mycobacterial 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, phenotypic drug susceptibility testing (DST) is required to confirm efficacy of empiric therapy and this may take additional days to weeks for results after the isolation and identification of the pathogens, depending on the method.

Explanation of the test

cobas® MAI for use on the **cobas**® 5800/6800/8800 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**® 5800/6800/8800 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**® 5800/6800/8800 Systems. The **cobas**® 5800 System is designed as one integrated instrument. 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**® 5800 or **cobas**® 6800/8800 Systems 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 (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

The materials provided for **cobas**® MAI can be found in Table 1. All unopened reagents and controls must be stored as recommended in Table 1 to Table 4. Materials required, but not provided can be found in Table 2 through Table 4, and Table 8 through Table 10.

Table 1 cobas® MAI

cobas® MAI

Store at 2-8°C
384 test cassette (P/N 09040595190)

Kit components	Reagent ingredients	Quantity per kit
Proteinase Solution (PASE)	Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase, glycerol 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-MAI 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

For use on the **cobas**® 5800 System (P/N 09040609190)

For use on the **cobas**® 6800/8800 Systems (P/N: 07544863190 or P/N 09040609190)

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® Buffer Negative Control Kit

cobas® Buffer Negative Control Kit

Store at 2-8°C

For use on the **cobas®** 5800 System (P/N 09051953190)

For use on the **cobas®** 6800/8800 Systems (P/N 07002238190 or P/N 09051953190)

Kit components	Reagent ingredients	Quantity per kit
cobas® 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: Harmful if swallowed. H314: Causes severe skin burns and eye damage. H411: Toxic to aquatic life with long lasting effects. EUH032: Contact with acids liberates very toxic gas. EUH071 Corrosive to the respiratory tract. P273: Avoid release to the environment. P280: Wear protective gloves/protective clothing/eye protection/face protection/ hearing 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. P391 Collect spillage. 593-84-0 guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol
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 8 to Table 10).
**Product safety labeling primarily follows EU GHS guidance
***Hazardous substance or mixture.

Reagent storage and handling requirements

Reagents must be stored and handled as specified in Table 5, Table 6 and Table 7.

When reagents are not loaded on the **cobas**® 5800 or **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

Table 5 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

Reagent handling requirements for the cobas® 5800 System

Reagents loaded onto the **cobas**® 5800 System are stored at appropriate temperatures and their expiration is monitored by the system. The system allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 5800 System.

Table 6 Reagent expiry conditions enforced by the **cobas**® 5800 System

Reagent	Kit expiration date	Open-kit stability	Number of runs for which this kit can be used	On-board stability
cobas® MAI	Date not passed	90 days from first usage	Max 40 runs	Max 36 days[b]
cobas® MAI Positive Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 36 days[b]
cobas omni Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a] Single use reagents.

[b] Time is measured from the first time that reagent is loaded onto the **cobas**® 5800 System.

Reagent handling requirements for the cobas® 6800/8800 Systems

Reagents loaded onto the **cobas**® 6800/8800 Systems are 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	Kit experiation date	Open-kit stability	Number of runs for which this kit can be used	On-board stability (cumulative time on board outside refrigerator)
cobas® MAI	Date not passed	90 days from first usage	Max 40 runs	Max 40 hours
cobas® MAI Positive Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas® Buffer Negative Control Kit	Date not passed	Not applicable[a]	Not applicable	Max 10 hours
cobas omni Lysis Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni MGP Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Specimen Diluent	Date not passed	30 days from loading[b]	Not applicable	Not applicable
cobas omni Wash Reagent	Date not passed	30 days from loading[b]	Not applicable	Not applicable

[a] Single use reagents

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

Additional materials required for the cobas® 5800 System

Table 8 Materials and consumables for use on the **cobas**® 5800 System

Material	P/N
cobas omni Processing Plate 24	08413975001
cobas omni Amplification Plate 24	08499853001
cobas omni Liquid Waste Plate 24	08413983001
Tip CORE TIPS with Filter, 1mL	04639642001
Tip CORE TIPS with Filter, 300µL	07345607001
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 or Solid Waste Bag With Insert	07435967001 or 08030073001

Additional materials required for the cobas® 6800/8800 Systems

Table 9 Materials and consumables for use on the **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

Table 10 Other materials and consumables required for pre-analytic workflow

Materials
cobas® Microbial Inactiviation Solution (P/N 08185476001)
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**® 5800/6800/8800 Systems - User Assistance and/or User Guides. 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**® 5800 software and **cobas**® MAI analysis package for the **cobas**® 5800 System must be installed on the **cobas**® 5800 instrument. The Data Manager software and PC for the **cobas**® 5800 System will be provided with the system.

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

Table 11 Instrumentation

Equipment	**P/N**
cobas® 5800 System	08707464001
cobas® 6800 System (Option Moveable)	06379672001
cobas® 6800 System (Fix)	05524245001
cobas® 8800 System	05412722001
Sample Supply Module	06301037001

Refer to the **cobas**® 5800 System or **cobas**® 6800/8800 Systems – User Assistance and/or User Guides for additional information.

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 and adquate risk assessment as outlined in Biosafety in Microbiological and Biomedical Laboratories, in the CLSI Document M29-A4 and in the Tuberculosis Laboratory Biosafety Manual by WHO.[16-18] Only personnel proficient in handling infectious materials and the use of **cobas**® MAI and **cobas**® 5800/6800/8800 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.
- Sample liquefaction and mycobacterial inactivation by MIS should be performed in a biological safety cabinet (BSC) within a Biosafety Level B3[18] in line with local and institutional guidelines[16] or regulations and based on an adequate risk assessment.
- 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.
- If spillage of samples in MIS occurs, FIRST clean with a suitable laboratory detergent and water, and then with 70% ethanol.
- 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 contamination of samples is not adequately controlled during sample handling and processing.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Inform your local competent authority about any serious incidents which may occur when using this assay.

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, **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) within a Biosafety Level B3[18] or other biosafety control environment according to local and institutional guidelines[16] or regulations and based on an adequate risk assessment.
- 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, **cobas**® MAI Positive Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** reagents, and consumables 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 0.6% sodium or potassium hypochlorite in distilled or deionized water . Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 5800/6800/8800 Systems, follow the instructions in the **cobas**® 5800 or **cobas**® 6800/8800 Systems - User Assistance and/or User Guides 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**® 5800/6800/8800 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**® 5800/6800/8800 Systems - User Assistance and/or User Guides 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**® 5800/6800/8800 Systems.
- Refer to the **cobas**® 5800/6800/8800 Systems - User Assistance and/or User Guides for proper maintenance of instruments.

Prior to running MAI on the **cobas**® 5800/6800/8800 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 specimens". Abbreviated representative workflows are summarized in Table 12 for the raw sputum specimen type and in Table 13 for the sediment specimen type. For further details, refer to the subsequent sections.

Note: Specimen inactivation by MIS should be performed in a biological safety cabinet (BSC) within a Biosafety Level B3[18] or other biosafety measures in line with local and institutional guidelines or regulations and based on an adequate risk assessment.

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 12 Workflow overview - Raw sputum specimen type

BSL-3 (BSC)	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**® 5800 or **cobas**® 6800/8800 Systems and start run using the raw sputum specimen type

Table 13 Workflow overview - Sediment specimen type

BSL-3 (BSC)	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**® 5800 or **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 14.

- 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 14.

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

  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 14 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**® 5800/6800/8800 assays using the same specimen type or for repeat testing.

Sonication of specimens

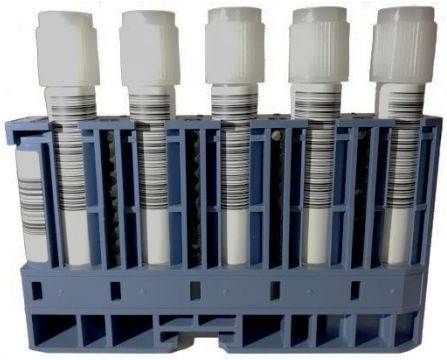
- Sonication of specimens for running **cobas**® MAI must be performed using the tube sonicator TS 5 device from Rinco Ultrasonics AG (P/N 46690). The use of other sonication devices may lead to false positive, false negative and/or invalid results. The operation of the sonicator is described in detail 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 on the cobas® 5800 System

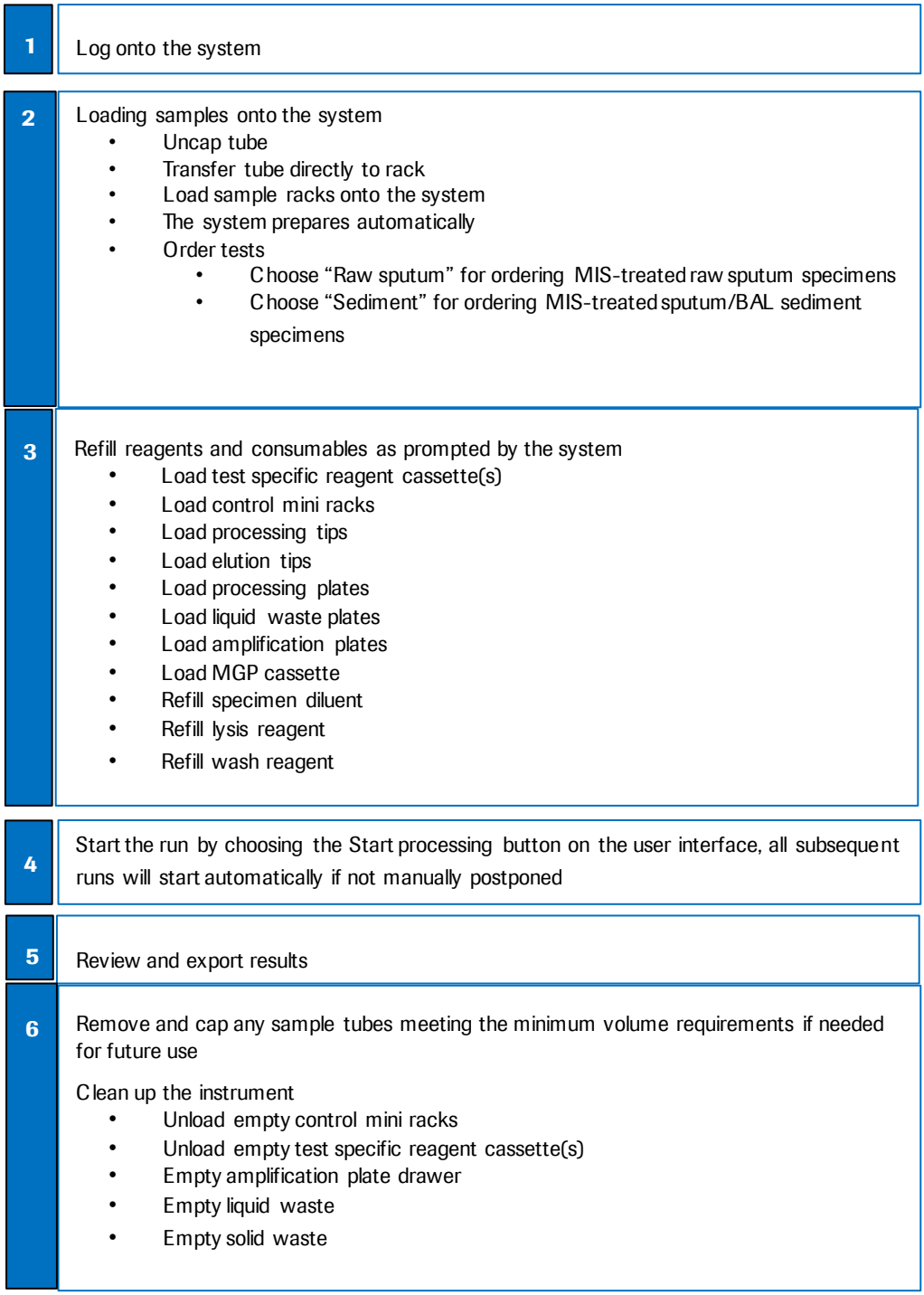
cobas® MAI can be run with a minimum sample volume of 1.2 mL of which 850 µL is processed. The test procedure is described in detail in the **cobas**® 5800 Systems - User Assistance and/or User Guide. Figure 2 below summarizes the procedure.

- Prior to uncapping tubes and loading specimens onto the **cobas**® 5800 System, 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.
- A single run can have a combination of specimens (raw sputum, sediment).

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**® 5800 System.

Figure 2 **cobas**® MAI test procedure on the **cobas**® 5800 System



Running cobas® MAI on the cobas® 6800/8800 Systems

cobas® MAI can be run with a minimum required sample volume of 1.2 mL of which 850 µL is processed. The operation of the instrument is described in detail in the **cobas**® 6800/8800 Systems - User Assistance and/or User Guides. Figure 3 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.
- A single run can have a combination of specimens (raw sputum, sediment).

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 3 **cobas**® MAI procedure on the **cobas**® 6800/8800 Systems

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 on the cobas® 5800 System

- One negative control [(-) Ctrl] and one positive control [MAI (+) C] are processed at least every 72 hours and with every new kit lot. Positive and/or negative controls can be scheduled more frequently based on laboratory procedures and/or local regulations.
- In the **cobas**® 5800 software and/or report, check for flags and their associated results to ensure the result validity.

Invalidation of results is performed automatically by the **cobas**® 5800 software based on negative or positive control failures.

NOTE: The **cobas**® 5800 System will be delivered with the standard setting of running a set of controls (positive and negative) with every run, but can be configured to a less frequent scheduling up to every 72 hours based on laboratory procedures and/or local regulations. Please contact your Roche service engineer and/or Roche customer technical support for more information.

Control results on the cobas® 5800 System

The results of the controls are shown in the **cobas**® 5800 software in the "Controls" app.

- Controls are marked with "Valid" in the column "Control result" if all targets of the control are reported valid. Controls are marked with "Invalid" in the column "Control result" if all or one target of the control are reported invalid.
- Controls marked with "Invalid" show a flag in the "Flags" column. More information on why the control is reported invalid including flag information is shown in the detail view
- If one of the controls is invalid, repeat testing of all controls and all associated samples is required.

Quality control and validity of results on the cobas® 6800/8800 Systems

- One negative control [(-) Ctrl] and one 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 Assistance and/or User Guide.
- 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 Systems software based on negative and positive control performance, and validation of individual sample results is performed by the **cobas**® 6800/8800 Systems software based on internal control results.

Interpretation of results

Results and their corresponding interpretation for detecting MAI are shown in Table 15.

Table 15 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.

Interpretation of results on the cobas® 5800 System

The results of the samples are shown in the **cobas®** 5800 software in the "Results" app.

For a valid control batch, check each individual sample for flags in the **cobas®** 5800 software and/or report. The result interpretation should be as follows:

- Samples associated with a valid control batch are shown as 'Valid' in the "Control result" column if all control target results reported valid. Samples associated with a failed control batch are shown as 'Invalid' in the "Control result" column if all control target results reported invalid.
- If the associated controls of a sample result are invalid, a specific flag will be added to the sample result as follows:
    - Q05D : Result validation failure because of an invalid positive control
    - Q06D : Result validation failure because of an invalid negative control
- The values in "Results" column for individual sample target result should be interpreted as shown in Table 15 above.
- If one or more sample targets are marked with "Invalid" the **cobas®** 5800 software shows a flag in the "Flags" column. More information on why the sample target(s) is reported invalid including flag information is shown in the detail view.

Figure 4 Example of **cobas**® MAI results on the **cobas**® 5800 System

Sample ID	Test	Control result	Flag	Status	Result		Creation date/time
MIA_S_pos-02	MAI	Valid		Released	**MIN Positive (Ct 38.51)**	**MAV Positive (Ct 39.27)**	6/30/2022 1:33:50 PM
MAI_S_pos-01	MAI	Valid		Released	**MIN Positive (Ct 37.15)**	**MAV Positive (Ct 37.42)**	6/30/2022 1:33:51 PM
MAI_S_neg-02	MAI	Valid		Released	MIN Negative	MAV Negative	6/30/2022 1:33:52 PM
MAI_S_neg-01	MAI	Valid		Released	MIN Negative	MAV Negative	6/30/2022 1:33:51 PM
MAI_S_inv-01	MAI	Valid		Released	**MIN Invalid**	**MAV Invalid**	6/30/2022 1:33:53 PM
MAI_RS_pos-02	MAI	Valid		Released	**MIN Positive (Ct 38.72)**	**MAV Positive (Ct 38.61)**	6/30/2022 1:33:51 PM
MAI_RS_pos-01	MAI	Valid		Released	**MIN Positive (Ct 37.39)**	**MAV Positive (Ct 37.49)**	6/30/2022 1:33:51 PM

Interpretation of results on the cobas® 6800/8800 Systems

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 Systems 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.

Figure 5 Example of **cobas**® MAI results on the **cobas**® 6800/8800 Systems

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

Procedural limitations

- **cobas**® MAI should always be performed along with mycobacterial 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**® 5800/6800/8800 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**® 5800/6800/8800 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 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 10 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 10 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 performed on the cobas® 6800/8800 Systems

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 256 CFU/mL and 241 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 16 and Table 17. 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 18 and Table 19) yielded overall CV (%) ranging from 1.5% to 2.7% for *M. intracellulare* and from 1.5% to 2.5% for *M. avium*.

Table 16 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 17 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 18 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 19 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 20 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 20 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 21). 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 21 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 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-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 22 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 archived specimens (raw sputum, sputum/BAL sediments) from subjects with presumptive mycobacterial respiratory infection prospectively 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 mycobacterial 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 23.

Table 23 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%]
PPV	**Raw Sputum**	**MIN PCR+**	MIN	16/16 **100.0%** [79.4-100%]	N/A
		MAV PCR +	MAV	27/27 **100.0%** [87.2-100%]	N/A
		MIN a/o MAV PCR +	MIN/MAV	44/44 **100.0%** 92.0-100%]	N/A
	Sediment	**MIN PCR +**	MIN	27/27 **100.0%** [87.2-100%]	32/36 **88.9%** [73.9-96.9%]
		MAV PCR +	MAV	35/35 **100.0%** [90.0-100%]	36/37 **97.3%** [85.8-99.9%]

				Roche **cobas® MAI**	Roche **COBAS® TaqMan® MAI**
		MIN a/o MAV PCR +	MIN/MAV	62/62 **100.0%** [94.2-100%]	68/73 **93.2%** [84.7-97.7%]
NPV	**Raw Sputum**	**MIN PCR -**	MIN	350/358 **97.7%** [95.6-99.0%]	N/A
		MAV PCR -	MAV	350/354 **98.9%** [97.1-99.7%]	N/A
		MIN a/o MAV PCR -	MIN/MAV	350/361 **96.7%** [94.6-98.5%]	N/A
	Sediment	**MIN PCR -**	MIN	412/431 **95.6%** [93.2-97.3%]	408/422 **96.7%** [94.5-98.2%]
		MAV PCR -	MAV	412/435 **94.7%** [92.2-96.6%]	**411/433** **94.9%** [92.4-96.7%]
		MIN a/o MAV PCR -	MIN/MAV	412/454 **90.7%** [87.7-93.3%]	407/443 **91.9%** [88.9-94.2%]

C = Culture, MIN = *Myconbacterium intracellulare*, MAV = *Mycrobacterium avium*, a/o = and/or

System equivalency / system comparison

System equivalency of the **cobas®** 5800, **cobas®** 6800 and **cobas®** 8800 Systems was demonstrated via performance studies. The results presented in the Instructions for Use support equivalent performance for all systems.

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

Symbols

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

Table 24 Symbols used in labeling for Roche PCR diagnostics products

Age/DOB Age or Date of Birth	Device not for near-patient testing	QS IU/PCR QS IU per PCR reaction, use the QS International Units (IU) per PCR reaction in calculation of the results.
SW Ancillary Software	Device not for self-testing	
Assigned Range [copies/mL] Assigned Range (copies/mL)	Distributor *(Note: The applicable country/region may be designated beneath the symbol)*	SN Serial number
Assigned Range [IU/mL] Assigned Range (IU/mL)	Do not re-use	Site Site
EC REP Authorized representative in the European Community	Female	Procedure Standard Standard Procedure
BARCODE Barcode Data Sheet	For IVD performance evaluation only	STERILE EO Sterilized using ethylene oxide
LOT Batch code	GTIN Global Trade Item Number	Store in dark
Biological risks	Importer	Temperature limit
REF Catalogue number	IVD In vitro diagnostic medical device	TDF Test Definition File
CE CE marking of conformity; this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device	LLR Lower Limit of Assigned Range	This way up
	Male	Procedure UltraSensitive Ultrasensitive Procedure
Collect Date Collect date	Manufacturer	UDI Unique Device Identifier
Consult instructions for use	CONTROL − Negative control	ULR Upper Limit of Assigned Range
Σ Contains sufficient for <*n*> tests	NON STERILE Non-sterile	Urine Fill Line Urine Fill Line
CONTENT Content of kit	Patient Name	Rx Only US Only: Federal law restricts this device to sale by or on the order of a physician.
CONTROL Control	Patient number	Use-by date
Date of manufacture	Peel here	
Device for near-patient testing	CONTROL + Positive control	
Device for self-testing	QS copies / PCR QS copies per PCR reaction, use the QS copies per PCR reaction in calculation of the results.	

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Technical support

For technical support (assistance) please reach out to your local affiliate:
https://www.roche.com/about/business/roche_worldwide.htm

Manufacturer

Table 25 Manufacturer

Manufactured in the United States

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

Made in USA

Trademarks and patents

See https://diagnostics.roche.com/us/en/about-us/patents

Copyright

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Document revision

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The summary of safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed