EUCAST European Committee on Antimicrobial Susceptibility Testing

Public Consultation

Proposed "Working protocol for determining MIC of amikacin, clarithromycin and azithromycin on reference strains of *Mycobacterium avium* ATCC 700898 and *Mycobacterium abscessus* ATCC19977, using the EUCAST Reference protocol for MIC determination of anti-mycobacterial agents against isolates of nontuberculous mycobacteria (NTM)

Annexe document

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Please send comments to the EUCAST Scientific Secretary at Mandy.Wootton@wales.nhs.uk by DATE 17 April 2026

see the main document

Introduction

All laboratories willing to test the protocol on one or both reference strains will follow the protocols detailed in the "EUCAST Reference protocol for MIC determination of anti-mycobacterial agents against isolates of nontuberculous mycobacteria (NTM)" main document and also below.

For the two species, clarithromycin, azithromycin and amikacin are tested.

- For *Mycobacterium avium* ATCC 700898, the protocol is that described for SGM, i.e. in cation-adjusted Mueller-Hinton broth (CAMH) supplemented with 5% OADC and plates with incubation at 36°C ± 1°C
- For *Mycobacterium abscessus* ATCC 19977, the protocol is that described for RGM, i.e. in CAMH without OADC and plates with incubation at 30°C ± 1°C

Preparation of the drugs is done as follows:

- Follow the protocol A from the Reference protocol (main document) for amikacin in sterile water. An example is provided in Table S1.
- Follow the protocol B from the Reference protocol (main document) for clarithromycin and azithromycin, dissolved in DMSO and tested in 0.5% DMSO final. An example is provided in Table S2.

Reading dates for the MIC:

- D3, D5, and D14 (clarithromycin and azithromycin) for *M. abscessus*
- D7, D10 and D14 for *M. avium*

For assessing quality control using the working protocol, testing is done

- 4 times on the same day (4 plates a day, testing repeatability on the same culture)
- with 4 repeats done on separate days (4 different days performed on four different culture, testing reproducibility).

A total of 16 plate results will be obtained.

Results

MIC results should be compiled and compared for repeatability (results of the same day and same inoculum preparation) and reproducibility (results from different days and different inoculum preparations). MIC values should be within the range of the published QC and close to the target MIC.

Table S1. Preparation of the two range of concentrations of amikacin (water soluble agent) for testing the reference protocol for *M. abscessus* (without OADC) and *M. avium* (with OADC) reference strains.

Antimicrobial	e.g. Supplier/lot number	Solvent	Stock conc (mg/L)	Working conc. (4X) in CAMH +/- OADC* (mg/L)	Final concentration in CAMH +/- OADC (mg/L)
Amikacin	A1774	dH_2O	10 240	4096	Low range 64-0.5 High range 1024-8

CAMBH, cation-adjusted Mueller-Hinton broth.
*5%OADC for *M. avium* representative of slowly growing mycobacteria and no OADC for *M. abscessus* representative of rapidly growing mycobacteria

Table S2. Preparation of clarithromycin and azithromycin (non-water soluble anti-mycobacterial agents) two ranges of concentrations in DMSO (0.5% final) for testing the reference protocol for *M. abscessus* (without OADC) and *M. avium* (with OADC) reference strains.

Antimicrobial	e.g. Supplier/lot number	Solvent	200X stock solutions (mg/L) prepared in 100% DMSO	2X working solutions (mg/L) prepared by adding 10 µL of stock solution to 990 µL CAMH +/- OADC	Final concentration (mg/L) in CAMH +/- OADC containing 0.5% DMSO
Clarithromycin	Sigma A3487	DMSO	25600 - 12	256-0.12	Low range 16-0.06 High range 128-1
Azithromycin	Sigma/Merck PHR1088	DMSO	51200 - 25	512-0.25	Low range 32-0.12 High range 256-2

CAMBH, cation-adjusted Mueller-Hinton broth

*5%OADC for *M. avium* representative of slowly growing mycobacteria and no OADC for *M. abscessus* representative of rapidly growing mycobacteria