SENSITITRE®

Broth Microdilution (MIC) Method:

For Rapidly Growing Mycobacteria (RGM), Slowly Growing Nontuberculosis Mycobacteria, Nocardia and other Aerobic Actinomycetes

For Research Use Only

For full plate information, including plate layout, QC information refer to www.trekds.com/techinfo. The plate code and batch number will be required.

INTENDED USE

Susceptibility testing of rapidly growing mycobacteria including *Mycobacterium fortuitum* group (*M. fortuitum, M. peregrinum, M. fortuitum* third biovariant complex), *M. chelonae*, *M. abscessus, M. mucogenicum)* and *M. smegmatis* group (*M. smegmatis, M. goodii, M. wolinskyi*). *Nocardia* spp and other aerobic actinomycetes. Slowly growing nontuberculosis mycobacteria (NTM), i.e. *Mycobacterium avium* complex, *Mycobacterium kansasii* and *Mycobacterium marinum.*

Please refer to CLSI for details of testing *M. marinum*

TREK Diagnostic Systems manufactured broth has only been validated with Sensititre® Products.

PRINCIPALS OF USE

Each plate is dosed with antimicrobial agents at appropriate dilutions. Results can be read manually by visual reading of growth.

PRECAUTIONS

Only personnel trained and qualified in susceptibility testing techniques should use the system. **The laboratory should have established biosafety guidelines for handling mycobacteria.**

STORAGE AND SHELF LIFE

The plates should be stored at room temperature (15-25°C) away from direct sunlight and direct heat. Each plate is packaged in foil with a silica gel desiccant. Do not use the plate if past its expiration date, or the desiccant colour is not blue or orange or the foil pouch is damaged.
Inoculate plate within 5 hours of removal from pouch.

PROCEDURE

Materials included:

Sensititre® plate
Adhesive seal

Materials not included: [TREK Inc Product Code]

Sensititre® demineralized water [T3339]
Sensititre® cation adjusted Mueller-Hinton broth with TES buffer (CAMHBT) [T3462]
Sensititre® cation adjusted Mueller-Hinton broth with OADC [T8006]
Sensititre® doseheads (for use with AutoInoculator® / AIM®) [E3010]
Sensititre® AutoInoculator® [V3010]
Sensititre® AIM® [V3020]
Sensititre® Vizion® [V2021]
Manual Viewer [V4007]
0.5 McFarland turbidity polymer standard [E1041]
50µL or 100µL pipettor and disposable tips
Quality control strains
Agar plates
Incubator 30-35^{0}C, non-CO_2
Vortex mixer
Demineralized water with glass beads
Current CLSI documents

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected, transported, stored and then plated on to primary isolation medium to give isolated colonies using standard procedures.

SELECTION OF SUSCEPTIBILITY TEST BROTH

Use Sensititre® CAMHBT for rapid growing mycobacteria, *Nocardia* and other aerobic Actinomycetes or Mueller Hinton broth with OADC for slow growing mycobacteria.
Sensititre® broths are performance tested for use with Sensititre® susceptibility products.

INOCULATION AND INCUBATION

Rapid growing mycobacteria, *Nocardia* spp and other aerobic actinomycetes

Allow all broths to come up to room temperature before use.

1. Sweep the confluent portion of growth from growth on an agar plate with a swab. Emulsify in demineralized water and adjust to a 0.5 McFarland Standard visually or using the Sensititre® nephelometer. If particles are visible, vortex well. Actinomycetes typically have very hard crusty colonies. It may be necessary to vortex with glass beads to make a homogeneous suspension. If large clumps remain after vortexing, they should be allowed to settle and the supernatant used for the inoculum suspension.

Moistening the swab with demineralized water may facilitate a more homogeneous solution…

Warning- the use of water supplemented with Tween may affect MICs

2. Transfer 50µl of the suspension into a tube of cation adjusted Mueller-Hinton broth with TES buffer to give an inoculum of $5x10^5$ cfu/mL (range $1x10^5$ to $1x10^6$ cfu/mL). Mix well.

Steps 1 and 2 must be completed within 30 minutes.

3. Plates containing ≥ 32µg/mL doxycycline or minocycline may show a precipitate after incubation. This can be prevented by reconstituting these well with 5µL sterile distilled water before addition of broth.
4. Transfer 100µL to each well by either:
a. **Sensititre® AutoInoculator® / AIM®.** Replace the tube cap with a Sensititre® single-use dosehead and inoculate the plate according to the AutoInoculator® instructions.

Remove the test tube/dosehead combination from the AutoInoculator® / AIM® within 30 seconds of dosing a plate and store inverted in a rack or discard.

b. **Manual pipette.** Pour the broth into a sterile seed trough and inoculate the plate using an appropriate pipette. Dose the panel with the Sensititre® label facing the user.
Pipettes should be periodically serviced and checked for calibration

Inoculate broth into a plate within 30 minutes.

5. A periodic check of the colony count of the positive control well should be done. (See Appendix 1) Isolates should have an inoculum of $5x10^5$ CFU/mL, (range $1x10^5$ – $1x10^6$)

6. Cover all wells with the adhesive seal. Press all wells firmly to assure adequate sealing. Avoid creases as these can lead to skips.

7. Incubate rapid growing mycobacteria aerobically at 30^0C in a non-CO^2 incubator for 72 hours. Check for growth. If poor, reincubate for up to a further 48 hours. Incubate *Nocardia* and other aerobic Actinomycetes at 35^0C in a non-CO^2 incubator for 2-3 days.

Incubation of 4-5 days may be required for isolates of *M. chelonae* and *M. abscessus* (1)

CLSI recommends nonpigmented rapidly growing Mycobacteria be incubated for 14 days to ensure detection of inducible macrolide resistance, unless the isolate is resistant at an earlier reading.

Plates can be stacked up to three high.

Slow Growing Nontuberculous Mycobacteria (NTM including MAC)
Same procedure as above except transfer 50µL of the organism suspension into 11mL of Sensititre® Mueller-Hinton broth with 5% v/v OADC growth supplement. Invert the tube 8-10 times. It may help to vortex with saline with glass beads to make a homogeneous suspension.

Incubate at 35^0C in a non-CO_2 incubator and read after 7 days. If growth is good in the positive control, read results. Otherwise re-incubate for up to 14 days. CLSI M24 provides reading guidelines and illustrations of various growth patterns. Prolonged incubation may require taking steps to prevent loss of well contents through evaporation. Place the plates in a plastic container with the top ajar to facilitate gas exchange.

READING TEST RESULTS

Results can be read using the Sensititre® manual viewer or the Vizion®. See User Vizion® Manual. It is not necessary to remove the adhesive seal. Place the plate with the label facing the user. Growth appears as turbidity or as a deposit of cells at the bottom of a well. The MIC is recorded as the lowest concentration of antimicrobic that inhibits visible growth. Please refer to CLSI M24 for guidance on reading endpoints. Reading faint growth on Vizion® can be improved by use of bright indirect lighting against a dark background.

The positive growth control wells should be read first. If any show no growth, results are invalid.

Mycobacteria end points can be difficult to interpret. CLSI M24 provides reading guidelines and illustrations of various growth patterns. Negative wells can show a slight precipitate related to the inoculum. Reading QC strains with known MICs should be used for training

Growth can range from a few colonies with no turbidity to heavy growth comparable to positive growth control. The MIC is the lowest concentration that completely inhibits growth except for sulphonamides, where the MIC is read as the lowest concentration that inhibits 80% growth compared to the positive control.

The following points should be noted:

a. Contamination

Contamination may result in growth in a well bordered by wells showing no growth. Such a single well contamination can be ignored, but if multiple well contaminants are suspected, the test should be repeated.

b. Skips

Occasionally a “skip” may be seen - a well showing no growth bordered by wells showing growth. There are variety of explanations including contamination, mutation, creased seal and misaligned dosing. A single skip can be ignored. However, in order to ensure effective antimicrobic therapy NEVER read the skipped well as the MIC; always read the lowest well concentration above which there is consistently no growth.

c. Mixed Cultures

Except as referred to in (a) above, if two end points are seen as a distinct “button” of cells followed by several wells of diffuse growth with the “button” no longer visible (or seen as smaller buttons), there may be a mixed bacterial population. Purity should be checked by sub-culturing growth onto suitable agar. Test results are invalid if a mixed culture is detected.

INTERPRETATIVE GUIDELINES

Refer to the MIC Interpretive guidelines as provided by the CLSI, EUCAST or your national reference group.

INDICATIONS FOR USE

Antimicrobial	RGM	NOC.	SGM [1]	SGM [2]	MAC
Amikacin	X	X	XX	X	
Amoxicillin/ Clavulanic acid	XX	X			
Capreomycin					
Cefepime		XX			
Cefoxitin	X				
Cefotaxime		XX			
Ceftriaxone		X			
Ciprofloxacin	X	X	XX	X	
Clarithromycin[7]	X	X	XX[3]	X	X
Clofazimine[4]	X			X	X
Doxycycline	X	XX			
Ethambutol			XX	X	
Ethionamide					
Gentamicin		XX			
Imipenem	X	X			
Isoniazid[5]			XX	XX	
Kanamycin					
Levofloxacin	X				
Linezolid	X	X	XX	X	
Meropenem	XX	XX			
Minocycline	X	X	XX	X	
Moxifloxacin	X	X	XX	X	
Ofloxacin[6]					
Rifabutin			XX	X	
Rifampin			X	X	
Streptomycin			XX	XX	
Sulfamethoxazole	X	X	XX	XX	
Tigecycline	X				
Tobramycin	X	X			
Trimethoprim/ Sulfamethoxazole	X	X	XX	X	

Key
RGM Rapid growing mycobacteria
NOC *Nocardia* spp.
SGM Slow growing mycobacteria
MAC *M.avium* complex

X: First line
XX: Second line

[1] Information on *M.kansasii*
[2] Slow growers other than MAC or *M.kansasii*
[3] Clarithromycin is the only antimicrobial reported for *M.avium* complex. Hence it is a first line drug for this organism
[4] Requires a single patient IND to obtain clofazimine in the USA. Susceptibility methods and breakpoints have not been established or standardized by CLSI.
[5] Problematic for testing NTM; breakpoints have yet to be established for NTM.
[6] May not be available in the USA.
[7] The final reading for nonpigmented rapidly growing mycobacteria should be at 14 days to ensure detection of inducible macrolide resistance, unless the isolate is resistant at an earlier reading.

QUALITY CONTROL

Frequency of quality control testing should be according to local guidelines. Inocula should be cultured on a suitable medium to check for purity and/or colony morphology composition [a]. Test results are invalid if a mixed culture is detected.

All Sensititre® plates include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells

A number of factors influence MIC's including organism state, inoculum density, temperature and broth. In practice, replicate MIC's form a normal distribution with the majority of results lying within one dilution of the modal value. The test procedure can be considered satisfactory if control organism MIC's are within range. Results should **not** be reported if QC results are not in range.

Table 1 lists tentative QC ranges using mycobacteria strains. Until other data becomes available, *S.aureus* ATCC 29213 and other non-mycobacterial QC strains and ranges from CLSI document M100 can be used for panel QC (Table 2).

CLSI M100 QC strains should use the same inoculation method as for rapid growing mycobacteria except that 50µL of inoculum should be added to Sensititre® Mueller Hinton broth with TES. Do not use broth supplemented with OADC. Panels should be read after 18 to 24 hours incubation at 35°C.

M. avium 700898 QC strain should be incubated at 35^0C in a non-CO_2 incubator and read at 7 days. If growth is good in the positive control, read results. Otherwise re-incubate for up to 10 days. CLSI M24 provides reading guidelines and illustrations of various growth patterns.

[a] Morphological variation has been observed with *M. avium* 700898. Up to three colony types have been identified (1. Smooth dome, 2. Opaque dome with transparent rough edge, 3. Flat and transparent with rough edge). MIC may be affected by colony type(s) used for inoculum. MIC of Isoniazid, Moxifloxacin and Rifampin may vary between *M. avium* 700898 cultures.

Table 1:
Tentative Quality Control Ranges for mycobacteria QC strains[1] and *S.aureus* ATCC 29213

Antimicrobial agent	*S.aureus* ATCC 29213	*M.peregrinum* ATCC 700686	*M.smegmatis* ATCC 19420	*M.avium* ATCC 700898
Amikacin		≤1 – 4*	0.12 - 0.5	2-16
Amoxicillin/clavulanate 2:1	0.12/0.06-0.5/0.25			
Capreomycin	-	1.25 – 10	1.25 – 5	5 – 40
Cefotaxime	1-4			
Cefepime	1-4			
Cefoxitin	1-4*	4-32*		
Ceftriaxone	1-8			
Ciprofloxacin	0.12-0.5*	≤ 0.12 – 0.5*	0.25-1 **	2-16[3]
Clarithromycin	0.12-0.5*	≤ 0.06 – 0.5*		0.25-4[3]
Clofazimine	-	0.25 – 1	0.12 - 0.5	–
Doxycycline	0.12-0.5*	0.12 – 0.5*		≥2[3]
Ethambutol	-	2-16		2-16
Ethionamide	-	>20	20-80****	0.6 – 5 (7 days incubation) 1.25-10 (10 – 11 days incubation)
Gatifloxacin	0.03-0.12	≤ 0.12**	≤ 0.12**	
Imipenem	0.015-0.06*	2 – 16*		
Isoniazid	-		1-4	≥1[3]
Kanamycin	1-4	1.25 – 10	≤ 0.06	1.25 – 10
Levofloxacin	0.06-0.5	0.12 – 0.5***	0.12 – 0.5***	
Linezolid	1-4*	1-8*		8-32[3]
Meropenem	0.03-0.12*	2-16*		
Minocycline	0.06-0.5[4]	0.12-0.5*		
Moxifloxacin	0.015-0.12	≤0.06-0.25*		0.25 – 4[3]
Ofloxacin	0.12-1	≤ 0.25 – 0.5	0.25 – 1	
Rifabutin (Ansamycin)	-	1-8	1 – 4	≤0.25-1[3]
Rifampicin	0.004-0.015	8-64	>16	≥1[3]
Streptomycin	-	16 – 64	0.5 – 2	4-32
Sulphamethoxazole	32-128*[2]	≤ 1 – 4*		
Tetracycline	0.12-1			
Tigecycline	0.03-0.25			
Tobramycin	0.12-1*	2-8*		
Trimethoprim / sulphamethoxazole	≤ 0.5/9.5*	≤0.25/4.8-2/38*		0.25/4.8-2/38

Notes [1] CLSI M100 QC range (2)

[2] The MIC range listed is for sulfizoxazole for *S.aureus*
[3] Ranges based on Sensititre® in house testing; MIC with *M. avium* 700898 may be affected by colony morphology type.
* CLSI M24QC range (1)
** Range based on data from Reference 3
*** Range based on data from Reference 4
**** Incubation time dependent. Range based on 72 hours incubation only.

Table 2:
Additional QC ranges[1]

Antimicrobial agent	*E.faecalis* ATCC 29212	*E.coli* ATCC 25922	*P.aeruginosa* ATCC 27853	*E.coli* ATCC 35218
Amikacin		0.5-4	1-4	
Amoxicillin/clavulanate 2:1	0.25/0.12-1/0.5	2/1-8/4		4/2-16/8
Cefotaxime		0.03-0.12	8-32	
Cefepime		0.015-0.12	1-8	
Cefoxitin		2-8		
Ceftriaxone		0.03-0.12	8-64	
Ciprofloxacin	0.25-2	0.004-0.015	0.25-1	
Doxycycline	2-8	0.5-2	4-32[2]	
Gatifloxacin	0.12-1	0.008-0.03	0.5-2	
Imipenem	0.5-2	0.06-0.25	1-4	
Kanamycin		1-4		
Levofloxacin	0.25-2	0.008-0.06	0.5-4	
Linezolid	1-4			
Meropenem	2-8	0.008-0.06	0.25-1	
Minocycline	1-4	0.25-1		
Moxifloxacin	0.06-0.05	0.008-0.06	1-8	
Ofloxacin	1-4	0.015-0.12	1-8	
Rifabutin (Ansamycin)		4-16[2]		
Rifampicin	0.5-4	4-16	16-64	
Streptomycin		4-16[2]		
Tetracycline	8-32	0.5-2	8-32	
Tigecycline	0.03-0.12	0.03-0.25		
Tobramycin	8-32	0.25-1	0.25-1	
Trimethoprim/ sulphamethoxazole	≤ 0.5/9.5	≤ 0.5/9.5	8/152-32/608	

[1] CLSI M100 QC range (2) unless otherwise stated
[2] Range based on Sensititre® in-house testing

Contact Sensititre® Distributor or TREK Diagnostic Systems in the event that quality control discrepancies cannot be resolved.

PERFORMANCE

Panels are designed to give comparable performance to CLSI reference micro-broth procedures (1). Performance has been independently investigated (references 5-7)
For further information contact TREK Diagnostic systems or your local distributor.

LIMITATIONS

1. Imipenem results for *M.chelonae* and *M.abscessus* should not be reported (1)
2. Tobramycin is the aminoglycoside of choice for *M. chelonae* and should only be reported for this organism (1)

APPENDIX 1: Colony Count Procedure

1.Immediately following inoculation plate, using a 1µl loop, sample from the positive growth control well and inoculate onto an appropriate agar.

2.Take another loop (1µl) and sample from the same growth well and mix with 50µl sterile deionised water. Inoculate 1µl of this dilution onto an appropriate agar plate to obtain countable colonies.

3.Incubate both plates at 30 or 35 ^{0}C (depending on type of organism) over night .

4.Read as follows:

Number of colonies on plate

Colony Count	**0.001 plate**	**0.001of 1/50 dilution**
$<5 \times 10^{4} =$	<50	0
$5 \times 10^{4} - 1X10^{5} =$	50 – 100	0 –2
$1 \times 10^{5} - 5 \times 10^{5} =$	100 – 500	≤10
$> 5 \times 10^{5} =$	>500	>10

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