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Instructions for Use

Thermo Scientific™ Sensititre™
MIC Susceptibility Plates
for Rapidly Growing Mycobacteria (RGM), Slowly Growing Non-tuberculosis Mycobacteria, Nocardia and other Aerobic Actinomycetes

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MIC Susceptibility Plates
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For *Research Use Only*

For full plate information, including plate layout, QC information, please 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,* and the former *M. fortuitum* third biovariant complex now identified to species level., e.g.*M. porcinum, M. seneglense, etc,*, *M. chelonae* (e.g., *M. chelonae, M. immunogenum, m. saopaulense)*, *M. abscessus* complex (*Mycobacterium abscenssus,* subsp *abscessus* subsp *massiliense* and subsp *bolletii),*the *M. mucogenicum* group(e.g., *M. mucogenicum, M. phocaicum, m. aubagnense)* and *M. smegmatis* group (*M. smegmatis, M. goodii, M. wolinskyi*). *Nocardia* spp and other aerobic actinomycetes. Slowly growing nontuberculosus mycobacteria (SGNTM), i.e. *Mycobacterium avium* complex, *Mycobacterium kansasii* and *Mycobacterium marinum,* etc.

Thermo Scientific manufactured Sensititre panels have only been validated with Sensititre broth.

SUMMARY AND PRINCIPLES OF USE

Each plate is dosed with antimicrobial agents at appropriate dilutions. Results can be read manually by visually observing for 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 or broth if past its expiration date, or the desiccant color is not orange, or the foil pouch is damaged.

Inoculate plate within 5 hours of removal from pouch

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected, transported, stored and then plated on to primary isolation medium according to established microbiological laboratory practice to give isolated colonies.
(Reference: Manual of Clinical Microbiology 12th ed.)

PROCEDURE

Materials included:

- 10 Sensititre plates
- 10 Adhesive seals

Materials not included [Inc Product Code]:

- Sensititre™ demineralized water [T3339]
- Sensititre™ demineralized water with glass beads [T3493]
- 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 AIM™) [E3010]
- Sensititre™ AIM™ [V3020]
- Sensititre™ Vizion™ [V2021]
- Sensititre™ Nephelometer [V3011]
- Sensititre™ static cling panel seals [G523M]
- Manual Viewer [V4007]
- 0.5 McFarland turbidity polymer standard [E1041]
- 50µL or 100µL pipettor and disposable tips
- Quality control organisms
- Agar plates
- Incubator 30-35^{0}C, non-CO_2
- Vortex mixer
- Current CLSI, EUCAST, or local guideline documents

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 PROCEDURE

A) 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 AIM.** Replace the tube cap with a Sensititre single-use dosehead and inoculate the plate according to the AIM instructions.

Remove the test tube/dosehead combination from the 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 rapidly growing mycobacteria aerobically at 30^0C in a non-CO^2 incubator for 72 hours. Check for growth. If poor (≤1+), re-incubate 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. A few species may require up to 5 days for adequate growth in broth. The exception is *Rhodococcus equi* which is incubated for 24 hours.

Incubation of 4-5 days may be required for isolates of *M. chelonae* and *M. abscessus* (1), however antimicrobials such as carbapenems and tetracyclines may be adversely affected (i.e. false resistance after extended incubation). Therefore, if incubation exceeds 4 days, this should be noted on the report.

CLSI recommends MIC's of Clarithromycin with rapidly growing mycobacteria be incubated up to 14 days to ensure detection of inducible macrolide resistance, unless the isolate is resistant at an earlier reading or molecular testing has identified the presence or absence of a functional erm gene.

Plates can be stacked up to three high.

B) Slowly 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 demineralized water with glass beads to make a homogeneous suspension.

Incubate at 35^{0}C in a non-CO_2 incubator (*M. marinum* is incubated at 30^{0}C) and read after 7 days. If growth is good (≥2+) in the positive control, read results. Otherwise re-incubate for up to 14 days checking periodically. 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. Pinpoint growth in wells above the MIC should be disregarded.

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.

INTERPRETATION OF RESULTS

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	X
Amoxicillin/ Clavulanic acid		X			
Cefepime		XX			
Cefoxitin	X				
Ceftriaxone		X			
Ciprofloxacin[8]	X	X	XX	X	
Clarithromycin[7]	X	X	XX[3]	X	X
Clofazimine[4]	X			X	X
Doxycycline	X	XX			
Imipenem	X	X			
Isoniazid[5]			XX	XX	
Linezolid	X	X	XX	X	XX
Meropenem	XX	XX			
Minocycline	X	X	XX	X	
Moxifloxacin	X	X	XX	X	XX
Rifabutin			XX	X	
Rifampin			X	X	
Streptomycin[9]			XX	XX	
Tigecycline[11]	X				
Tobramycin[12]	X	X			
Trimethoprim/ Sulfamethoxazole	X	X	XX	X	

Key
RGM Rapidly growing mycobacteria
NOC *Nocardia* spp.
SGM Slowly 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] Clofazimine Susceptibility methods and breakpoints have not been established or standardized by CLSI. MIC's are given without interpretations.
[5] Problematic for testing NTM; breakpoints have yet to be established for NTM.
[7] The final reading for rapidly growing mycobacteria should be at 14 days to ensure detection of inducible macrolide resistance, unless the isolate is resistant at an earlier reading.
[8] Ciprofloxacin is the class agent for levofloxacin and ofloxacin but not moxifloxacin. CLSI M24 3rd ed.
[9] Breakpoints have not been addressed by CLSI
[11] For RGM there are no breakpoints for Tigecycline
[12] Tobramycin for RGM is only reported for *M. chelonae/immunogenum* complex

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.

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 4).

Newly recommended QC isolates and ranges have been added in tables 1, 2 and 3.

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 14 days. CLSI M24 provides reading guidelines and illustrations of various growth patterns. If strain is not readable at 7 days replacing strain is recommended.

[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.
CLSI Recommendations is to test only transparent colonies of MAC. CLSI M24 3rded.
This strain is no longer recommended by CLSI as a routine QC isolate.

CLSI M24 3rded. Replaced *M. avium* 700898 with *M. marinum* ATCC 927 to allow for appropriate QC for agents other than clarithromycin and amikacin with other SGM.

Table 1. Broth Microdilution QC Ranges When Testing Slowly Growing NonTB Mycobacteria according to CLSI M62

	MIC QC Ranges, µg/mL*		
Antimicrobial agent	*M. marinum*†* ATCC 927	*S. aureus** ATCC 29213	*M. avium*[1*] ATCC 700898
Amikacin	1-4	1-4	2-16
Ciprofloxacin	1-8	0.12-0.5	
Clarithromycin	0.5-2	0.12-0.5	0.25-4
Clofazimine	0.03-0.25**	2-8**	
Doxycycline	1-8	0.12-0.5	
Linezolid	1-4	1-4	8-32
Minocycline	-	0.06-0.5	
Moxifloxacin	0.5-4	0.015-0.12	0.25-4
Rifabutin	≤0.12-0.5	-	
Rifampicin	≤0.5-2	0.004-0015	
Streptomycin	8-32	-	
Trimethoprim/ sulfamethoxazole	≤ 0.5/9.5-4/16	≤ 0.5/9.5	

*QC ranges were established using unpublished data collected from studies performed by three CLSI development committee members.
Inoculum: Sensititre Mueller-Hinton broth with 5% v/v OADC growth supplement (T8006)
** Sensititre development range
†*M. marinum* should be incubated at 30°C ±2°C for 7 days.
1. Supplemental QC for when testing *Mycobacterium avium complex* (MAC)

Table 2. Broth Microdilution QC Ranges When Testing Rapid Growing NonTB Mycobacteria according to CLSI M62

Antimicrobial agent	*M. peregrinum*† ATCC 700686	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853	*E. faecalis* ATCC 29212
Amikacin	≤1-4	1-4	1-4	64-256
Cefoxitin	4-32	1-4	-	-
Ciprofloxacin	≤0.12-0.5	0.12-0.5	0.25-1	0.25-2
Clarithromycin	≤0.06-0.5	0.12-0.5	-	-
Clofazamine	0.12-1[1]	2-8[1]		
Doxycycline	0.12-0.5	0.12-0.5	-	2-8
Imipenem	2-16	0.015-0.06	1-4	0.5-2
Linezolid	1-8	1-4	-	1-4
Meropenem	2-16	0.03-0.12	0.25-1	2-8
Minocycline	0.12-0.5	0.06-0.5	-	1-4
Moxifloxacin	≤0.06-0.25	0.016-0.12	1-8	0.06-0.5
Tigecycline	0.03-0.25	0.03-0.25	-	0.03-0.12
Tobramycin‡	2-8	0.12-1	0.25-1	8-32
Trimethoprim/ sulfamethoxazole	≤ 0.25/4.8-2/38	≤ 0.5/9.5	8/152-32/608	≤ 0.5/9.5

†*M. peregrinum* should be incubated at 30°C ±2°C for 3 days.
‡ Report for *M. chelonae/immunogenum* only
1. Sensititre Development Range

Table 3. Broth Microdilution QC Ranges When Testing *Nocardia* spp. And Other Aerobic Actinomycetes according to CLSI M62

Antimicrobial agent	*N. nova* ATCC BAA-2227	*S. aureus* ATCC 29213	*E. coli* ATCC 35218	*E. faecalis* ATCC 29212
Amikacin	≤1	1-4		
Amoxicillin/Clavulanate	8/4-128/64	0.12/0.06-0.5/0.25	4/2-16/8	
Ceftriaxone	2-16	1-8	-	-
Ciprofloxacin	2-16	0.12-0.5		
Clarithromycin	0.03-0.25	0.12-0.5		
Doxycycline	1-8	0.12-0.5		
Imipenem	0.25-2	0.016-0.06		
Linezolid	0.5-2	1-4		
Minocycline	1-8	0.06-0.5		
Moxifloxacin	1-8	0.016-0.12		
Rifampin‡		0.004-0.016		0.5-4
Trimethoprim/ sulphamethoxazole	0.12/2.4-2/38	≤ 0.5/9.5		
Tobramycin	4-64	0.12-1		
Vancomycin		0.5-2		1-4

‡ Report for R. equi only.

Table 4: Additional QC ranges[1]

Antimicrobial agent	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Amikacin		0.5-4	1-4
Amoxicillin/clavulanate 2:1	0.25/0.12-1/0.5	2/1-8/4	
Cefepime		0.015-0.12	1-8
Cefoxitin		2-8	
Ceftriaxone		0.03-0.12	8-64
Ciprofloxacin		0.004-0.015	
Doxycycline		0.5-2	
Imipenem		0.06-0.25	
Linezolid			
Meropenem		0.008-0.06	
Minocycline		0.25-1	
Moxifloxacin		0.008-0.06	
Rifabutin (Ansamycin)		4-16[2]	
Rifampin	0.5-4	4-16	16-64
Tigecycline		0.03-0.25	
Tobramycin		0.25-1	
Trimethoprim/ sulphamethoxazole		≤ 0.5/9.5	

[1] CLSI M100 QC range (2) unless otherwise stated

Contact Sensititre Distributor or Thermo Fisher Scientific Technical Services for assistance in the event that quality control discrepancies cannot be resolved. See final page for contact information.

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. Tobramycin is the aminoglycoside of choice for *M. chelonae M. immunogenum complex* and should only be reported for this complex (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 x 10 ^{4} =	<50	0
5 x 10 ^{4} –1X10^{5} =	50 – 100	0 –2
1 x10 ^{5} – 5 x 10 ^{5} =	100 – 500	≤10
> 5 x 10 ^{5} =	>500	>10

REFERENCES

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10. CLSI M24, 3rd ed.

11. CLSI M62Ed1E

12. CLSI M100Ed30E

DISCLAIMER

The information provided in this technical insert is current at the time of printing and may change without notice.
Reference CLSI M24, 3rd ed.
Reference CLSI M62Ed1E

Any change or modification of the instructions may affect results. Thermo Fisher Scientific will not be liable for any damages resulting from any changes to storage, precautions, handling or testing procedures of the current version of instructions.

The latest information can be downloaded from the **www.TREKDS.com/techinfo** or by contacting Thermo Fisher Scientific Microbiology Technical Support .

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