



Sensititre® 20-24 hour *Haemophilus influenzae*/ *Streptococcus pneumoniae* MIC Susceptibility Plates and JustOne® strips

For *in vitro* Diagnostic Use

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

INTENDED USE

The Sensititre® *Haemophilus influenzae*/ *Streptococcus pneumoniae* plates and JustOne® strips are *in vitro* diagnostic products for clinical susceptibility testing of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Streptococcus species*.

SUMMARY AND PRINCIPALS OF USE

Each plate is dosed with antimicrobial agents at appropriate dilutions. Results can be read manually by visual reading of growth or automatically on an ARIS® / Autoreader® using fluorescence. The Sensititre Autoreader® system utilizes fluorescence technology. The technology involves the detection of bacterial growth by monitoring the activity of specific surface enzymes produced by the test organism. Growth is determined by generating a fluorescent product from a non-fluorescent (fluorogenic) substrate. The non-fluorescent substrate is prepared by conjugating a fluorescent compound to the specific enzyme substrates with a bond, which prevents fluorescence. The fluorophore, is then said to be quenched. The plates are prepared with the substrate already added to the plate (SIW). Enzymatic action of the bacterial surface enzymes on the specific substrates cleave this bond releasing the fluorophore, which is now capable of fluorescence. The amount of fluorescence detected is directly related to the activity of the bacterial surface enzymes and, therefore, to the bacterial growth.

Streptococcus pneumoniae and *Streptococcus* spp plates can either be read manually or automatically on the Sensititre Autoreader® and/or ARIS®. *H. influenzae* can only be read manually on the Vizion® or manual viewer.

JustOne® strips

Refer to the format (in configuration sheet enclosed separately) before using the strips. The JustOne® strip is a single row of 12 wells containing the antimicrobial dilutions along with one positive control well. The positive control well on each strip contain no antimicrobic and are used to check for satisfactory growth in the broth system used. The JustOne® strip is equal to one row of the Sensititre MIC susceptibility plate and can be read manually.

PRECAUTIONS

Results should be used as an aid to selecting the drug of choice for treatment.

Only personnel trained in susceptibility testing techniques should use the system.

Since living micro-organisms used with this product can be infectious to the user, proper handling and disposal methods should be used.

In the United States Federal law restricts this device to sale, use and distribution by or on the order of a physician or clinical laboratory.

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 colour is not blue 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

PROCEDURE

Materials included:

Sensititre[®] plate with substrate (SIW) or without substrate (Non-SIW) in wells

Sensititre[®] JustOne Strip

Adhesive seal

Materials not included [TREK Inc Product code]:

Sensititre[®] cation adjusted Mueller-Hinton broth with TES buffer and lysed horse blood (CAMHBT+ LHB) for inoculating plates read manually [CP-112]

Sensititre[®] cation adjusted Mueller-Hinton broth with TES buffer and lysed horse blood (CAMHBT+ LHB) for inoculating plates read automatically [CP-114]

Sensititre[®] cation adjusted Mueller-Hinton broth with TES buffer (CAMHBT) [T3462-05]

Sensititre[®] Haemophilus Test Medium (HTM) [T3470]

Sensititre[®] doseheads (for use with AutoInoculator[®]) [E3010]

Sensititre AutoInoculator[®] [V3010] or Sensititre AIM™ [V3020-CE]

Sensititre AutoReader[®] [V3029] or ARIS[®] [V3090]

Sensititre Vizion[®] [V3020]

Sensititre Nephelometer [V3011]

Manual viewer [V4007]

0.5 McFarland polymer turbidity standard [E1041] or 0.5 McFarland barium sulphate turbidity standard [E1040]

1ul and 10µl calibrated loops

50 or 100µl pipettor and disposable tips

Quality control strains

Agar plates

Incubator 34-36°C, non-CO₂

Vortex mixer

Current CLSI documents

SELECTION OF SUSCEPTIBILITY TEST BROTH

All Sensititre[®] approved broths are performance tested for use in Sensititre[®] susceptibility products.

INOCULATION PROCEDURES FOR SENSITITRE[®] PLATES and JUSTONE[®] STRIPS

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

1. For all plates and JustOne[®] strips transfer Pick 3-5 colonies from fresh primary agar plate, from an overnight chocolate agar plate (*H. influenzae*) or sheep blood agar plate (*Streptococcus* spp.) incubated in a 5% CO₂ atmosphere, into 5ml CAMHBT and adjust to a 0.5 McFarland Standard visually or using the Sensititre nephelometer. Mix well.

2. Transfer 100µl of *Streptococcus* spp., into 11ml CAMHBT+LHB or 50µl of *H. influenzae* into 11ml HTM to give an inoculum of 5x10⁵ cfu/ml. Note: A small amount of white sediment may be present in the bottom of CP114-10 tubes.

Steps 1 and 2 should be completed within 30 minutes.

3. Transfer 100µl of the broth suspension into the plate or JustOne[®] strip by either:

a. Sensititre Autoinoculator[®] / Sensititre AIM. Replace the tube cap with a Sensititre[®] single-use dosehead and inoculate the plate according to the Autoinoculator[®] instructions (cannot be used for JustOne[®] strips).

b. Manual pipette. Pour the broth into a sterile seed trough and inoculate the plate or JustOne[®] strip using an appropriate pipette.

Inoculate broth into the plate or JustOne[®] strip within 30 minutes.

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

4. Perform a purity check on all final inocula after inoculation

5. A periodic check of the colony count of the positive control well should be done (See Appendix 1).

Streptococcus spp., and *Haemophilus influenzae* should have an inoculum of 5x10⁵cfu/ml (range 2x10⁵ – 7x10⁵ cfu/ml)

This check is important for *Streptococcus* spp., as inocula can vary depending on the conditions of incubation of the primary plate.

6. Cover all wells with the adhesive seal. Avoid creases as these can lead to skips.

INCUBATION

All fastidious organisms should be incubated at 34-36°C either in the ARIS[®] or in a non-CO₂ incubator for 20-24 hours.

Up to 3 plates can be stacked if not incubated in the ARIS[®].

Summary of set up and incubation

Organism group	McFarland Suspension medium	Final Inoculum	Inoculum transfer	Broth	Plate Reconstitution	Incubation conditions	Incubate Hours
<i>Haemophilus influenzae</i>	MHB	5x10 ⁵ cfu/mL	50µL	HTM	100µL	34°C-36°C Non-CO ₂	20-24
<i>Streptococcus pneumoniae</i> / <i>Streptococcus spp</i>	MHB	5x10 ⁵ cfu/mL	100µL	MHB + LHB	100µL	34°C-36°C Non-CO ₂	20-24

READING TEST RESULTS

Check purity test plates. Results are invalid if a mixed culture is present.

1. Automatically

Streptococcus spp., plates may be read automatically on the AutoReader[®] or ARIS[®] according to the instructions in the Software Manual.

JustOne[®] strips cannot be read on the AutoReader[®] or ARIS[®].

2. Manually

After incubation, results can be manually read using the Sensititre[®] manual viewer, the Vizion[®]. See User's Manual. It is not necessary to remove the adhesive seal. 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 antimicrobial that inhibits visible growth. Reading faint growth on Vizion[®] can be improved by adjusting the lighting.

When reading *Streptococcus* spp., adjust the lighting as required to look for turbidity, haziness, or a deposit of cells. A small amount of sediment may be present in the bottom of the wells.

Turbidity with *H. influenzae* is slight, adjust the lighting for better contrast.

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

The following points should be noted:

a. Fading End Points

Most organism / antimicrobial combinations give distinct end points. With some combinations there may be a gradual fading of growth over 2 to 3 wells. The end points should be taken as the first well that inhibits visible growth, except sulphonamides when the MIC must be read as an 80 - 90% decrease in growth compared to the control well.

b. 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.

c. Skips

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

d. 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 can be checked by sub-culturing the growth onto suitable agar. Test results are invalid if a mixed culture is detected.

INTERPRETATION OF RESULTS - Refer to www.trekds.com/techinfo

There are antimicrobial agents included in this plate that have not been proven to be effective for treating infections for all organisms tested. Refer to the individual FDA approved pharmaceutical antimicrobial agent package insert for interpreting and reporting results of antimicrobial agents that have shown to be active against organism groups both *in vitro* and in clinical infections.

For automatically read results, the software applies interpretive criteria based on FDA guidelines.

QUALITY CONTROL – Refer to www.trekds.com/techinfo

Frequency of quality control testing should be set according to standard guidelines. Inoculum should be cultured onto a suitable medium to check for purity. Test results are invalid if a mixed culture is detected.

All Sensititre® plates and JustOne® strips include positive control wells. Tests are invalid unless there is distinct growth in all positive control wells. Some plate formats also include a “negative growth” well. This well is used for calibration of the AutoReader® and is not required for manual reading.

A number of factors influence MICs including organism state, inoculum density, temperature and broth. In practice, replicate MICs form a normal distribution with the majority of results lying within one dilution of the modal value.

The control organisms listed in the quality control tables are recommended for monitoring the susceptibility test procedure and checking the potency of antimicrobics each time a test is set up. Refer to the quality control tables for expected quality control results. The test procedure can be considered satisfactory if the susceptibility results obtained with the control organisms are within the stated ranges. Results should **not** be reported if QC results are outside the stated ranges.

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

LIMITATIONS

1. Sensititre® fastidious plates should not be incubated in a CO₂ incubator
2. The ability of the Sensititre® system to detect resistance to antimicrobics as shown below is unknown because resistant strains were not available at the time of comparative testing. If such a strain is observed, it should be submitted to a reference laboratory.

<i>H. influenzae</i>	<i>S. pneumoniae</i>
Amoxicillin / clavulanic acid	
Ampicillin / sulbactam	
Cefaclor	Cefaclor
Cefuroxime	Cefuroxime
Chloramphenicol	Gemifloxacin
Clarithromycin	Levofloxacin
Piperacillin/Tazobactam	Vancomycin
Rifampin	
Trimethoprim/sulphamethoxazole	

3. Performance of the Sensititre® *Haemophilus influenzae* / *Streptococcus* spp., plates and JustOne® strips have not been established for the following antimicrobics. Results should not be reported.

Antimicrobial Agent	Do not report results for <i>H. influenzae</i>	Do not report results for <i>Streptococcus</i> spp., other than <i>S. pneumoniae</i>	Do not report results for <i>S. pneumoniae</i>
Ampicillin		X	X
Ampicillin/Sulbactam		X	X
Cefaclor		X	
Cefdinir		X	
Cefixime		X	X
Cefpodoxime		X	X
Ciprofloxacin		X	X
Clarithromycin		X	
Clindamycin	X	X	
Daptomycin	X		X
Ertapenem		X	
Erythromycin	X		
Imipenem		X	X
Linezolid	X		
Lomefloxacin		X	X
Meropenem			
Penicillin	X		
Piperacillin/Tazobactam		X	X
Rifampin		X	X
Telithromycin		X	
Tigecycline	X		
Vancomycin	X		

4. Beta-lactamase testing for *H. influenzae* may be used to predict resistance to ampicillin. However, some ampicillin-resistant strains are Beta-lactamase negative.

5. The interpretation of susceptibility test results requires trained clinical personnel who should use judgement and knowledge prior to accepting test results.

6. Autoread results for *Streptococcus* spp., are only generated for those drugs listed

PERFORMANCE - Refer to www.trekds.com/techinfo

Plates read either manually or automatically are designed to give comparable performance to CLSI reference micro-broth procedure. Comparable performance is defined as $\geq 90\%$ agreement to within a doubling dilution of the reference MIC.

For further information contact TREK Diagnostic Systems or your local distributor.

APPENDIX 1: Colony Count Procedure.

1. Immediately following plate inoculation, using a 1µl loop, sample from the positive growth control well and inoculate onto a blood agar.
2. Take another loop (1µl), sample from the same growth well and mix with 50µl sterile deionised water. Inoculate 1µl of this dilution onto a blood agar plate to obtain countable colonies.
3. Incubate both plates at 34 –36°C over night under appropriate conditions.
4. Read as follows:

Number of colonies

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

REFERENCES – Refer to www.trekds.com/techinfo for additional references

1. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, M7 Approved Standard. The Clinical and Laboratory Standards Institute.
2. Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M100 The Clinical and Laboratory Standards Institute.

DISCLAIMER

The information provided in this technical insert is current at the time of printing and may change without notice.

The latest information can be downloaded from the www.TREKDS.com/techinfo or by contacting TREK Technical services. To be translated



Manufactured by TREK Diagnostic Systems
Units 17-19 Birches Industrial Estate, East Grinstead,
West Sussex, UK. RH19 1XZ, UK
Tel: +44-1342-318777

Distributed by TREK Diagnostic Systems,
982 Keynote Circle, Suite 6, Cleveland, Ohio 44131
Technical Service USA: 1 (800) 642-7029



036-FASTUSA_V1.0
Date Updated: 4 July2011