SENSITITRE®
SUSCEPTIBILITY PLATES FOR CAMPYLOBACTER

For Research and Veterinary 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

The Sensititre® susceptibility system is a micro broth method that provides qualitative (Susceptible or Resistant) and quantitative Minimum Inhibitory Concentration (MIC) results in a dried plate format. TREK Diagnostic Systems manufactured broth has only been validated with Sensititre® Products.

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.

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.
Only instruments supported by Sensititre i.e. a simple mirror viewer, Sensitouch, Vizion, Sensititre Autoreader , Optiread and ARIS must be used to report results with CE IVD and FDA cleared Sensititre products, any other system used will not be supported.

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 (Perforated at each well)

Materials not included [TREK Inc Product Code]:
Sensititre® cation adjusted Mueller-Hinton broth with TES buffer-5ml (CAMHBT) [T3462-5ml]
Sensititre® cation adjusted Mueller-Hinton broth with TES buffer and lysed horse blood (CAMHBT+ LHB) [CP 112]
Sensititre® dose heads (for use with AutoInoculator ®/ AIM®) [E3010]
Sensititre AutoInoculator® [V3010] / Sensititre AIM® [V3020]
Sensititre Vizion® [V2021]
Sensititre Nephelometer ® [V3011]
Manual viewer [V4007]
0.5 McFarland turbidity standard [E1041]
100µl pipette and disposable tips

Quality control strains
TSA w/5%sheeps blood Agar plates
Incubator ≥ 36^0C, (Microaerophilic environment required)
Vortex mixer
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® approved CAMHBT + LHB

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

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

Check the carton label for the reconstitution volume of the well.
A 100µl well inoculum is recommended for Campylobacter isolates.

1. Pick several colonies from a sheep blood agar plate incubated in a microaerophilic atmosphere for 48 hours at 36^0C or 24 hours at 42^0C into 5 ml Sensititre® CAMHBT and adjust to a 0.5 McFarland Standard visually or using the Sensititre® Nephelometer®. Mix well.

2. Transfer 100µl into 11ml Sensititre® CAMHBT + LHB to give an inoculum of $5X10^5$ CFU/ml.
Mix well.
3. Transfer 100µl by either:

a. **Sensititre AutoInoculator® / Sensititre AIM® .** Replace the tube cap with a Sensititre® single-use
dose head and inoculate the plate according to the AutoInoculator® /AIM® 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. Warning- Pipette tip must not contact reconstituted well content as this could lead to antibiotic carry over

N.B. A plate intended for 50µl may be dosed with 100µl but the resulting dilutions will be one doubling dilution lower.

4. 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 $1x10^5$ – $1 x 10^6$).

5. Cover all wells with a perforated adhesive seal. Avoid creases as these can lead to skips.

6. Provide a microaerophilic atmosphere for plate incubation Use sealable plastic pouches containing 10% CO_2, 5% O_2 and 85% N_2; or atmospheres generated by commercially available systems. Gas generators manufactured by Remel, Oxoid and Mitsubishi have been evaluated and perform well with Sensititre® Campylobacter susceptibility panels.

Steps 1 through 6 should be completed within 30 minutes.

INCUBATION

All plates should be incubated in a microaerohilic atmosphere at 42°C for 24 hours or 36-37^{0}C for 48 hours. Up to 3 plates can be stacked. Maintain a moist environment to prevent evaporation of well contents.

N.B. Use of 35^{0}C incubation has been inadequate for interpreting results at 24 hours. Do not exceed 43°C incubation.

Warning. Contents may leak in event that an inoculated panel is dropped. Disinfect appropriately according to laboratory practice procedures

READING TEST RESULTS

After incubation, results can be read using the Sensititre® manual viewer or the Vizion®. See Vizion® User 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 antimicrobic that inhibits visible growth. 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.

The following points should be noted:

a. Fading End Points

Most organism / antimicrobic 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 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 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 should be checked by sub-culturing growth onto suitable agar. Test results are invalid if a mixed culture is detected.

INTERPRETATION OF RESULTS

For interpretation of manually read results, refer to the MIC Interpretive guidelines as provided by the CLSI or your national reference group.

QUALITY CONTROL

Frequency of quality control testing should be according to local 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 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.

A number of factors influence MIC's including organism state, inoculum density, temperature, atmospheric conditions 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.

EXPECTED QC VALUES

	***C. jejuni* ATCC 33560**	
Incubation	**24hrs at 42°C**	**48hrs at 36-37°C**
Azithromycin	0.03 – 0.12	0.03 – 0.25
Ciprofloxacin	0.03 – 0.12	0.06 – 0.25
Clarithromycin	0.5 – 2	0.5 – 2
Clindamycin	0.12 – 0.5	0.12 – 1
Doxycycline	0.12 – 0.5	0.12 – 0.5
Erythromycin	0.25 –2	0.5-2
Florfenicol	0.5-2	1 - 4
Gentamicin	0.25 – 2	0.5 – 2
Levofloxacin	0.03-0.25	0.06-0.25
Meropenem	0.008-0.03	0.008-0.03
Nalidixic acid	4 – 16	4 – 16
Telithromycin	0.5 - 2	1 - 4
Tetracycline	0.25 – 1	0.25 – 2

Ranges have been established following CLSI M23 guidelines and have been approved by the CLSI Veterinary Subcommittee and will be published with the next revision of CLSI M31.

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

PERFORMANCE

Panels read manually are designed to give comparable performance to CLSI reference micro-broth procedure. For further information contact TREK Diagnostic systems or your local distributor

LIMITATIONS

1. Trained clinical personnel are necessary to make proper interpretations of test results.
2. In common with all other methods of antimicrobic susceptibility testing, the results generated by Sensititre® susceptibility plates are *in vitro* results.
3. Broth supplied by TREK has been specially formulated and quality controlled for reading panels.

APPENDIX 1: Colony Count Procedure

1. Immediately following inoculation of plate, using a 1µl loop, sample from the positive growth control well and inoculate onto a blood 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 a blood agar plate to obtain countable colonies.
3. Incubate both plates at 36 –37 ^{0}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 x 10	<50	0
5 x 10 ^{4} =	50 – 100	0 –2
1 x10 ^{5} – 5 x 10 ^{5} =	>100	≤10
> 5 x 10 ^{5} =	>100	>10

BIBLIOGRAPHY

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: M100 The Clinical and Laboratory Standards Institute.

3. CLSI M31. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard The Clinical and Laboratory Standards Institute (CLSI). 940 West Valley Road, Suit 1400, Wayne, PA190087, USA.

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 www.trekds.com\techinfo or by contacting TREK Technical services.

Manufactured by TREK Diagnostic Systems
Units 17-19 Birches Industrial Estate, East Grinstead,
West Sussex, 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