MIC Test Strip Technical Sheet **GRD**

GRD for Screening of hGISA/GISA

INTENDED USE

MIC Test Strip GRD (Glycopeptide Resistance Detection) is a double-sided predefined gradient of vancomycin (VA) and teicoplanin (TEC) for the detection of GISA (Glycopeptide Intermediate Staphylococcus aureus) or hGISA (hetero-GISA) phenotypes.
Any positive results for the MIC Test Strip GRD must be confirmed with further reference methods such us population analysis profiles.
MIC Test Strip GRD consists of a screening method for detecting GISA/hGISA phenotypes and can be tested with 0.5 McFarland and Mueller Hinton blood agar plates.

CONTENTS OF THE PACKAGES

The 10-test box contains 10 strips individually packed in desiccant envelops and an instruction sheet.
The 30-test box contains 30 strips individually packed in desiccant envelops and an instruction sheet.
The 100-test box contains 10 desiccant envelops, each containing 10 strips, and an instruction sheet. The 100-test box also contains a storage tube.

COMPOSITION

MIC Test Strip GRD strips are made of special featured paper carrier.
VA code indicates the vancomycin (0.5-32 µg/mL) gradient and TEC code indicates the teicoplanin (0.5-32 µg/mL).
The result from the GRD strip in combination with the standard vancomycin M.I.C. can be used to differentiate the GISA and hGISA phenotype.

GATHERING AND KEEPING SAMPLES

The colonies that are to test are taken up by culture media that have been previously swabbed with the sample under examination. In the case of mixed colonies the bacterial strains must be purified before inoculation.

TEST PROCEDURE

Before using MIC Test Strip GRD strips from an unopened package, visually inspect to ensure the package is intact.
Do not use the strips if the package has been damaged.
When removed from the -20 °C freezer, allow the package or storage container to reach room temperature for about 30 minutes.
Moisture condensing on the outer surface must evaporate completely before opening the package.

Materials required but not provided:

- Mueller Hinton II Agar + 5% Sheep Blood plates (ref. 10131)
- Mueller Hinton broth for preparing the inoculum
- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps
- 0.5 McFarland turbidity standard (ref. 80400)
- Incubator (35 ± 2 °C)
- Quality control organisms *S. aureus* ATCC® 29213 (MSSA), ATCC® 700699 (GISA) and ATCC® 700698 (hGISA).

Inoculum preparation

Suspend well-isolated colonies (various morphologies if present) from an overnight blood agar plate into Mueller Hinton broth to achieve a 0.5 McFarland standard turbidity.
A confluent or almost confluent lawn of growth will be obtained after incubation, if the inoculum is correct.
In order to verify that your procedure gives the correct inoculum density in terms of CFU/mL, performing regular colony counts is recommended.

Inoculation

Dip a sterile swab in the inoculum suspension and squeeze it on the wall of the test tube to eliminate excess liquid.
Alternatively, use a rotation plater to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed so that the surface is completely dry before applying MIC Test Strip GRD strips.

Application

Apply the strip to the agar surface with the M.I.C. scale facing upwards and code of the strip to the outside of the plate, pressing it with a sterile forceps on the surface of the agar and ensure that whole length of the antibiotic gradient is in complete contact with the agar surface. Once applied, do not move the strip.

Incubation

Incubate the agar plates in an inverted position at 35 ± 2 °C in ambient atmosphere.
Read after 18 - 24h and confirm results at 48h. Positive GISA/hGISA results after 18 - 24h can be reported.
Report negative results only after 48h.

EVALUATING THE RESULTS

Reading

Read the M.I.C. values for VA and TEC where the relevant inhibition ellipses intersect the strip, after a proper incubation time and only when an even lawn is clear.
The M.I.C. value is superior or equal to (≥) the highest value on the scale in case of no inhibition ellipse (growth along the entire gradient).
The M.I.C. value is inferior to (<) the lowest value when the inhibition ellipse intersects below the lower end of the scale.
In case of mutant colonies present in the inhibition ellipse, read the M.I.C. where those colonies are completely inhibited.
Inhibition ellipses may be small or not clearly determinable for high M.I.C. values.

Interpretation

GRD+ for GISA or hGISA VA or TEC ≥ 8 µg/mL
i) GISA: GRD+ and standard VA[1)] M.I.C. ≥ 4 µg/mL
ii) hGISA: GRD+ and standard VA M.I.C. <4 µg/mL

Note

1) The MIC Test Strip procedure for M.I.C. testing of VA (MIC Test Strip VA 0.016- 256 µg/mL) comprises Mueller Hinton agar, inoculum suspension in saline (0.5 McFarland) and incubation at 35 °C in ambient air for 24 hours. (refer to MTS-20: Staphylococci)

QUALITY CONTROL

Perform quality control using the recommended strains as described at TEST PROCEDURE to check the quality of the reagents and the procedure. The QC ranges (µg/mL) for MIC Test Strip GRD are listed below:

Strain	VA	TEC
S. aureus ATCC® 29213 (MSSA)	0.5-2	1-4
S. aureus ATCC® 700698 (hGISA)	1-8	≥32
S. aureus ATCC® 700699 (GISA)	4-16	≥32

PRECAUTIONS

The **MIC Test Strip** cannot be classified as being hazardous according to current legislation but fall within the specific field of application where a safety data sheet must be supplied because they can cause phenomena of sensitisation in sensitive subjects if they come into contact with the skin.
MIC Test Strip are disposable products. **MIC Test Strip** are only for diagnostic *in vitro* use and are intended for professional use. They must be used in the laboratory by properly trained operators using approved aseptic and safety methods for pathogenic agents.

STORAGE

All unopened packages and unused MIC Test Strip GRD strips must be stored at -20 °C or the temperature denoted on the package until the given expiry date. Unused strips must be stored in an airtight storage container with color indicating desiccant.
The batch number and expiry date should be clearly marked on the package and/or storage container.
Protect MIC Test Strip GRD strips from moisture, heat and direct exposure to strong light at all times.
Prevent moisture from penetrating into or forming within the package or storage container. MIC Test Strip GRD strips must be kept dry.

MIC Test Strip **GRD**: Glycopeptide Resistance Detection **investigational use only**

DESCRIPTION		µg/mL	CODE	packaging	REF.
MIC Test Strip	VANCOMYCIN / TEICOPLANIN	0.5-32/0.5-32	VA/TEC	10	921631
				30	92163
				100	921630

TABLE OF SYMBOLS

LOT	Batch code	**IVD**	*In Vitro* Diagnostic Medical Device		Manufacturer		Use by
REF	Catalogue number		Temperature limitation	Σ	Contains sufficient for <n> tests		Caution,consult accompanying documents

MIC Test Strip, Patent No. 1395483

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Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

MIC Test Strip Technical Sheet **Synergy Testing**

Specimen
Cystic fibrosis, multiple drug-resistant organisms, extreme drug resistant organisms, critical specimens, critical infections, critical patients, limited therapy options.

Procedure
Medium: See specific organism for appropriate agar media (e.g. MHA/aerobes, RPMI/fungi)
Inoculum: Suspension in saline (or broth) to 0.5 McFarland (ref.80400) or 1 McF (ref.80401) depending on bacteria. Inoculate normally by sterile swab.
Incubation: 35 ± 2 °C (or other) / ambient (or other) / 24-48 hours (or other) depending on the specific organism.
Interpretation of results: Bactericidal drugs: interpret the M.I.C. at complete inhibition of growth including microcolonies, hazes and isolated colonies. For bacteriostatic drugs, read at 80% inhibition when trailing is seen. When bactericidal is combined with bacteriostatic, read each agent according to their specific category.

Literature
MTS Pack insert, product labels, MTS Application Guide, MTS Interpretative Criteria and Quality Control , MTS Technical Sheets.

Definitions
MIC_A MIC of drug A alone
MIC_B MIC of drug B alone
MIC_{AB} MIC of drug A in combination with B
MIC_{BA} MIC of drug B in combination with A

Interpretation
Fractional Inhibitory Concentration Index (FIC Index) calculations:

FIC Index (Fractional Inhibitory Concentration Index) calculations:
FIC Index = $MIC_{AB}/ MIC_A + MIC_{BA}/ MIC_B$
MIC_{AB} = MIC of A in the presence of B; MIC_{BA} = MIC of B in the presence of A.

Interpretation	**FIC**
Synergy	≤ 0.5
Additive	> 0.5 and ≤ 1.0
Indifference	> 1 and ≤ 4.0
Antagonism	> 4.0

Results

	Single drug		Combination		FIC index	Interpretation
	MIC_A	MIC_B	MIC_{AB}	MIC_{BA}		
Strain 1						
Strain 2						

MTS Synergy Applicator System		
Product	**REF**	Σ
MTS Synergy Applicator Platform	96860	1
MTS Synergy Delivery Tool	96870	10 Tests

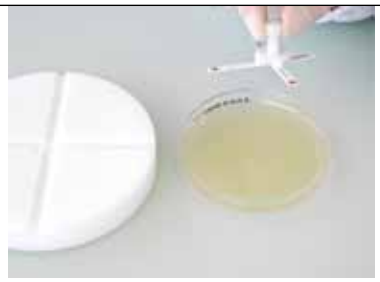
Download the video of the MTS Synergy Applicator System procedure: https://dl.dropbox.com/u/46181555/MTS%20crossing%20set.mov

Method

1. Perform standard M.I.C. of drugs A and B prior to synergy set-up.
2. Use the "MTS Synergy Applicator System"* for the synergy testing.
3. Take a MTS (MIC Test Strip) of the first antibiotic (A) with the tweezers and place it on the MTS Synergy Applicator Platform according to position 1.
4. Adjust the MTS (antibiotic A) such that the the MIC value of the first antibiotic (MIC_A) is positioned at the base intersection.

5. Take a MTS of the second antibiotic (B) with tweezers and place it on the base according to position 2.

6. Adjust the second MTS (antibiotic B) such that the MIC value of the second antibiotic (MIC_B) is positioned at the base intersection and intersects MTS-antibiotic A at its MIC value.



7. Use the MTS Synergy Delivery Tool, press hard onto the two carefully positioned MTS (A and B) and move them to the agar plate.

8. Carefully place the MTS Synergy Delivery Tool (with MTS_A and MTS_B) on the agar.
9. Wait until the strips are completely moistened by surface of the agar.

10. Remove the MTS Synergy Delivery Tool from the agar plate leaving MTS_A and MTS_B positioned at 90°. If necessary, use the tweezers to push the strips onto the agar surface.
11. Finally incubate according to the standard MTS procedure for the specific microorganism.

References

- CLSI M100-S22, 2012. Performance Standards for Antimicrobial Susceptibility Testing.
- CLSI M7-A9, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically.
- EUCAST. Breakpoint tables for interpretation of MICs and zone diameters Version 2.0, January 2012.

* MTS Synergy Applicator System *PATENT PENDING*: A device for standardising the *in-vitro* synergy testing of two antibiotics through the method of crossing the gradient strips. (Liofilchem, 2012).

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