MIC Test Strip Technical Sheet **GRD**

Vancomycin and Teicoplanin (VA/TEC)
For *in vitro* Glycopeptide resistance detection.

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).
- Additional technical information from www.liofilchem.net

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 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

After the required incubation period and only when an even lawn is distinctly visible, read the VA and TEC values* where the respective inhibition ellipses intersect the strip. Growth along the entire gradient i.e. no inhibition ellipse indicates that the value is greater than or equal to (≥) the highest value on the scale. An inhibition ellipse that intersects below the lower end of the scale is read as less than (<) the lowest value.

When mutant colonies are present in the inhibition ellipse, read the value where these colonies are completely inhibited. For values in the high range, inhibition ellipses may be very small or not clearly discernible.

* Important: the MIC Test Strip GRD should be used for confirmation of GRD production only and is not intended for the determination of the Minimum Inhibitory Concentration.

Interpretation

On GRD Strip, either VA or TEC ≥ 8 µg/mL indicate a positive result.

Then use standard Vancomycin strip (VA[1]) to distinguish between GISA and hGISA:

- GRD+ and standard VA ≥ 4 µg/mL indicates GISA;
- GRD+ and standard VA< 4 µg/mL indicates hGISA.

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 II Agar, inoculum suspension in saline (0.5 McFarland) and incubation at 35°C in ambient air for 24 hours. (refer to MTS20: 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

The unopened package of MIC Test Strip GRD should be stored at –20°C until the given expiry date. Leftover strips from an opened package must be stored at 2-8°C in the airtight tube, containing desiccant, provided in the pack for no more than 7 days. Do not store near sources of heat and do not expose to excessive temperature variations.

DESCRIPTION		µg/mL	Code	Packaging	Ref.
MIC Test Strip	Vancomycin/Teicoplanin	0.5-32/0.5-32	VA/TEC	10 30 100	921631 92163 921630

TABLE OF SYMBOLS

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

MIC Test Strip, Patent No. 1395483

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