RMIC™

Resistance Mechanisms Detection Minimum Inhibitory Concentration

Test for determination of Resistance Mechanisms Minimum Inhibitory Concentration
Product dedicated for professional studies for in vitro diagnostics of human and other origin samples.

PACKAGE SIZE

1 x 5 strips packed separately in foil sachets with a desiccant, in a carton box with instructions for use
1 x 10 strips packed separately in foil sachets with a desiccant, in a carton box with instructions for use
1 x 30 strips packed separately in foil sachets with a desiccant, in a carton box with instructions for use

INTRODUCTION

Testing with RMIC™ strips provides MIC values in µg/ml, indicating the amount of antibiotic that inhibits the growth of microorganisms under specified, standardized in vitro culture conditions. The strip is designed to achieve the highest repeatability of results, although slight variations in the obtained MIC value are possible, within plus or minus one twofold dilution (even with perfectly replicated test procedures). It should be noted that the indicated value is a minimum value; therefore, to effectively inhibit growth, an amount at least one value higher should be used. Many helpful insights can be found in the recommendation prepared by CLSI and EUCAST.

PURPOSE

The RMIC™ test is designed to determine the antimicrobial resistance (ESBL, MBL, GRD, AmpC and KPC). The strips have a the double-sided gradient of antibiotic applied, and a clear scale with markers allows for reading the MIC value in a twofold scale. The test is conducted on Petri dishes with a standardised agar medium, with the incubation time and conditions depending on the species being tested. RMIC™ allows for the examination of all species of microorganisms cultured on plates; the method of conducting the test may need to be adjusted. The test provides preliminary results, and further studies may be required to confirm the obtained results.

PRINCIPLE OF THE METHOD

RMIC™ strips are based on a combined method of microdilution and diffusion. Innovative strips, which have a defined constant gradient of antibiotic on their surface, allow for reading the MIC value on a twofold scale. The scale is designed to enable easy reading of both main values (twofold gradient) and intermediate values. On the top side, the strip in the logo area contains a two- or three-letter code of the antibiotic name; below this area is a clear scale with values expressed in µg/ml. The strips are sized to be used on standard Petri dishes (90mm and 150mm). It is possible to use two or more strips on one plate. The strip is designed to interact with the surface of the plate against the inoculated microorganisms and inhibit their growth proportionally to the resistance of the microorganism. Susceptibility can be determined by reading the MIC value in µg/ml from the scale, which is indicated by the elliptical-shaped inhibition zone; its narrow end intersects the scale on the strip at a specific point indicating the value. Detailed information on reading the values is provided below. The strips are designed to maintain durability and reproducibility of the obtained results. The user should verify the values obtained with a control strain to ensure accuracy. MIC values for specific strains and antibiotics are updated and included in recommendations prepared by EUCAST and CLSI.

STORAGE

1. Store the strips in their original packaging at the temperature indicated on the packaging (-20..8°C or -20°C). For long-term storage, temperatures below -20°C (+/- 2°C) are recommended.
2. Strips with the shortest expiration date must be used first.
3. Immediately before testing, bring the strips to room temperature (20°C; +/- 2°C). Do not heat the products with external heat sources; bring them to room temperature by taking the strips out of the freezer/refrigerator in advance.
4. Protect from moisture. The sealed packaging provides sufficient protection. An opened package with unused strips should be placed back into a sealed bag (container) along with a desiccant. Such a product should be used immediately, no later than a few days after opening. It must be stored under refrigeration (6°C; +/- 2°C) or frozen (-20°C; +/- 2°C).
5. Strips must be stored in a dry environment.
6. Do not use strips after expiry date.
7. If incorrect inhibition zones are observed during testing with control strains, verify the testing procedure. Incorrect results can be caused by poor storage, quality of the medium, or procedural errors.

PERFORMING THE TEST

After removing from the bulk packaging, check if the individual packages are not damaged. Do not use a strip if the packaging is damaged; use a new strip from undamaged packaging instead. Verify the expiration date and do not use strips that have passed their expiration date.

Before using the product, bring it to room temperature (see storage instructions). Remove the strips well in advance (approximately 30-60 minutes before testing). Check if moisture from the surroundings has settled on the outer packaging; if so, remove it with a paper towel. Contact with wet surfaces or direct exposure to liquids on the strip may cause improper functioning. A soaked strip should not be used; use a new one instead. Strips stored at room temperature can be used immediately.

Open the package by tearing at the top; the edge has a marker for easy opening. Remove the strip using tweezers, trying to grasp it in the logo area. Avoid touching the scale area; the antibiotic gradient is applied on its reverse side, and touching it may cause product malfunction. The tweezers should be dry and not soaked in sterilization solutions, or they should be dried before use. Other application tools can be used according to their usage instructions. You can touch the top side of the strip. The strip's special design protects the antibiotic gradient from interacting with the top side of the strip. After laying the strip, smooth it gently by sliding the tweezers along its top side; this will align its position and ensure optimal adhesion to the plate surface.

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Lay out the strips according to the test purpose, one, two, or more. When applying multiple strips, arrange them so that no edge of one strip touches another, leaving at least a 2 cm gap in the lower area of the strip (arranging them in a star pattern). If laying multiple strips side by side along a line, maintain a minimum gap of 3 cm between them. Arranging multiple strips with gradients of different antibiotics next to each other may complicate MIC value readings due to uneven microbial growth, synergy, or antagonism. Consider pharmacological interactions when analyzing the results obtained.

NOTES

RMIC™ strips are intended for professional use, in vitro diagnostic only.
RMIC™ strips should be used strictly according to the instructions and only for their intended purpose.
Strict adherence to aseptic procedures and personal protection measures is essential, following applicable regulations, especially when working with pathogenic microorganisms.
Waste generated from tests should be considered hazardous due to the presence of microorganisms and antibiotics. It is imperative to strictly follow regulations for the disposal of such waste, considering the potential for microorganisms to develop antibiotic resistance.
Materials from tests should not be stored longer than necessary, and measures should be taken to prevent the spread of resistant, potentially resistant, or antibiotic-resistant microorganisms into the environment.

PROCEDURE

Materials provided

5, 10, or 30 RMIC™ test strips individually packed in sachets with desiccant
Instructions for use

Materials required but not provided

Petri dishes with a minimum diameter of 90mm and special agar for the type of microorganisms being studied; detailed instructions are provided in Table 1
Suspension medium for preparing the inoculum; details are provided in Table 1
Sterile, non-toxic, moderately flexible swabs, tubes, scissors, pipette, tips, spreaders, and other small laboratory equipment
Densitometer
McFarland standards 0.5 and 1
Incubator (35°C; +/- 2°C), incubator with controlled CO2 atmosphere, or anaerostat depending on the strain being studied; details in Table 1
Reference strains

Culture medium

Adjust the culture medium to the specific microorganism being studied; details are found in Table 1. Ensure that the agar plates are uniform, providing suitable growth as per quality control for the plates; the agar must adhere well to the entire surface of the plates and their side walls. Bring the plates to room temperature before inoculation; do not heat them with an external heat source. Verify the expiration date; for comparative studies, use the same batch of plates for all tested samples. Follow the manufacturer's recommendations.

Preparation of Inoculum

Prepare the inoculum using the guidelines from Table 1. Use the standard method for preparing the inoculum according to EUCAST recommendations: pick several colonies from a pure, fresh culture to prepare the inoculum at the appropriate density compared to the standard. The inoculum should be prepared no earlier than 15 minutes before inoculation. Immediately before seeding, mix it to ensure uniformity and proper density for seeding. Inoculum tends to settle.

Inoculation

Place a sterile, flexible, non-toxic swab into the prepared inoculum by rotating it several times in the suspension. Remove excess solution by pressing the head (cotton swab) against the inner edge of the tube. Adjust the method according to the plates being seeded (size). Inoculate the plate with gentle, fluid movements to evenly cover the entire surface. After covering the plate, rotate it by 60° and repeat the seeding with the same swab; then repeat the process by rotating the plate another 60°. After spreading the suspension on the plate surface three times, gently circle around the plate's edge. Allow the plates to absorb the solution - wait approximately 15 minutes before placing the strips to ensure the plate surface is dry. The manufacturer recommends this seeding method, but other automatic or manual methods (spreading with a spreader) may also be used; however, ensure these methods provide a uniform lawn across the entire plate surface.

Warning:

The turbidimetric method does not guarantee the proper density of live cells in the inoculum. Perform a microbial count to verify the correct number of live cells in the inoculum. Properly prepared inoculum and seeding allow for homogeneous, consistent growth of microorganisms on the plate, enabling accurate reading of MIC values.

Application

Ensure that the inoculated plates are dry before application. Open the test sachet according to the instructions in the PERFORMING THE TEST section. Determine the best position on the plate, keeping in mind that the strips require a surface area of approximately 6 cm by 4 cm. Place one or a maximum of two strips containing the same antibiotic gradient on 90 mm plates. On 150 mm plates, you can place from one to six strips; the manufacturer recommends a maximum of 4 to 6 strips. Adjust the number of strips accordingly based on the expected size of the growth inhibition zones. If a large growth inhibition zone is expected (organism sensitive to the antibiotic), reduce the number of strips.

Strips can be applied using tweezers or other systems that apply suction or vacuum for handling, following the manufacturer's instructions. Ensure that the strip adheres well to the plate surface; if necessary, gently smooth the top of the strip with tweezers. If a strip improperly falls onto the agar surface, it may leave a mark that will be observed after incubation. Such a plate should be discarded or marked, and the user should assess whether they can correctly read the MIC value. If a strip is placed with the scale side facing the agar surface, correct results will not be obtained. Such a strip should be lifted and placed correctly with the scale side facing upwards. Such a plate should be discarded or left for the user's

assessment after incubation. The result may be difficult to read or incorrect. The manufacturer recommends repeating the test using a new inoculated plate and a new strip.

Incubation
Incubate the plates upside down according to the requirements of the tested microorganisms; guidelines are provided in Table 1. Follow the accepted standard methods of incubation.

Notes
Plates with agar used for testing may vary in parameters such as calcium and magnesium ion content, pH, even from the same supplier. Ion content and pH can affect the size of MIC values obtained. Use only verified suppliers and the same batch for control tests.
Read results after a specified incubation time; readings taken too early or too late may yield inaccurate results. Monitor the incubation time.
Incorrect incubation temperature, temperature instability, or interruptions in incubator operation may lead to erroneous results. Monitor the operation of the incubation devices; before reading results, ensure the device operated steadily during incubation.
Keep the user manual and refer to it if in doubt, or consult with the manufacturer.
Always conduct controls using reference strains, compare obtained results with available standards, and repeat the test if there are doubts. Users should validate the method for their specific needs and according to required standards.

Reading the results
RMIC™ BioMaxima strips have been specially designed for easy result interpretation. However, training and experience are required for more challenging or debatable cases. The innovative strips feature a scale and markers along the strip edge to facilitate reading. The result indicates the boundary between the zone of growth inhibition (surface without visible turbidity, single colonies – growth-free zone of microorganisms) and the surface where growth is observed. The arbitrary boundary line runs below the strip, with its ends touching the scale on both sides of the strip. This location indicates the MIC value for a given strain and antibiotic. Major values are placed on the scale for medical diagnostic purposes. The strip allows for intermediate values reading for research purposes or more accurate diagnostics. Intermediate values are read if the boundary between zones falls in the dark field between light fields. Major values are read if the boundary ends in a light area. For medical diagnostic purposes, if the boundary falls in a black area, read the value from the light field above the intermediate value (inflate the reading). If no elliptical or circular growth inhibition zone is observed, and even a minimal zone cannot be observed, read the maximum result indicated on the strip and indicate that the strain of microorganism is resistant to the antibiotic, express its resistance with the ">" symbol, and indicate the maximum value on the strip. If a large zone of growth inhibition is observed around the strip in such a way that the entire strip is surrounded by this zone, it means that the strain is sensitive to the antibiotic, indicate that the minimum inhibitory concentration is less than the minimum value on the strip. Several types of strips with different scales are available for different antibiotics, pay attention to the values placed on the strip. If single colonies resistant to the antibiotic are observed within the growth inhibition zone, indicate the value at which no colonies are present (total growth inhibition zone). Live microorganisms can exhibit different types of growth and characteristics of the obtained "lawn," therefore various types of deformations of the zone may occur in the form of funnels along the scale, or a thin layer of growth along the scale, in most cases they should be ignored. If the values read on both sides of the strip are different, indicate the higher value. In case of doubt, repeat the test. Consider the purpose of the study, as some antibiotics exhibit bacteriostatic properties, which cause partial growth inhibition zone to appear. Depending on the purpose, correctly indicate the MIC value. For bacteriostatics, indicate the MIC value at 80% of the growth inhibition zone, where the first significant reduction - growth limitation is observed by the naked eye. Bactericidal MIC values for a given antibiotic should be determined by indicating the boundary of total growth inhibition.

Interpretation of results
The interpretation of results should be performed by trained and experienced personnel. RMIC™ strips provide quantitative data, so values should be precisely indicated along with information on whether a particular strain is resistant or sensitive. Information regarding resistance is available in publicly accessible tables prepared by CLSI® and EUCAST. For control purposes, reference strains recommended by these organizations should be used. The tables specify values at which a strain should be considered resistant or sensitive. Tests have been designed to accurately indicate MIC values for reference strains. For medical diagnostic purposes, intermediate values should not be indicated but rounded up to the nearest higher value.

WASTE MANAGEMENT
All waste generated from tests using RMIC™ should be considered potentially infectious material. It should be noted that the strips contain antibiotics, and their use with microorganisms can lead to the development of resistance mechanisms against these antibiotics. Therefore, all waste should be disposed of immediately after the tests are completed to prevent them from entering the environment. It is the responsibility of laboratories to adhere to waste disposal regulations, implement appropriate procedures, and ensure compliance with them.

REFERENCES

1. World Health Organization Expert Committee on Biological Standardization. Requirements for antibiotic susceptibility test: 1.: WHO Technical reports series No 610. Geneva: WHO, 1977.
2. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0, January, 2024.
3. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; latest edition. CLSI supplement M100.
4. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; latest edition.CLSI standard M07.
5. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard, latestedition. CLSI document M11.

Table 1

Media, temperature, conditions and time of incubation recommended by EUCAST

Testing microorganisms	Medium	Incubation temperature	Incubation conditions	Incubation time
Enterobacterales	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Pseudomonas spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Stenotrophomonas maltophilia	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Burkholderia pseudomallei	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Acinetobacter spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Staphylococcus spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Enterococcus spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16-20 h; 24 h for glycopeptide
Aeromonas spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Achromobacter xylosoxidans	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Bacillus spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 18 h
Vibrio spp.	MH Agar (MH)	35 o C ± 1	Aerobic	16 – 20 h
Streptococcus spp. A,B,C,G Group	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Streptococcus pneumoniae	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Streptococcus spp. Viridans group	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Haemophilus influenzae	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Moraxella catarrhalis	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Listeria monocytogenes	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Pasteurella multocida	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5 % CO2	16 – 20 h
Campylobacter jejuni and coli	MH+5% KK+20 mg/l NAD (MH-F)	41 o C ± 1	microaerophilic conditions	24 h, up to 40-48 h
Corynebacterium spp.	MH+5% KK+20 mg/l NAD (MH-F)	35 o C ± 1	5% CO2	16 – 20 h, up to 40-44 h
Aerococcus sanguinicola and urinae	MH+5% KK+20 mg/l NAD (MH-F))	35 o C ± 1	5% CO2	16 – 20 h, up to 40-44 h
Kingella kingae	MH+5% KK+20 mg/l NAD (MH-F))	35 o C ± 1	5% CO2	16 – 20 h, up to 40-44 h
Brucella melitensis	MH+5% KK+20 mg/l NAD (MH-F))	35 o C ± 1	5% CO2	46 – 50 h
Anaerobes	Fastidious Anaerobic Agar (FAA)	35-37 o C ± 1	Anaerobic conditions	16 – 20 h

CE

Explanation of symbols

⚠	Caution, consult accompanying documents		Consult instructions for use		Manufacturer
IVD	For in vitro diagnostic use	LOT	Batch code	REF	Catalog number
	Temperature limitation		Use by		

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