Micropro® - MIC

Intended Use

Micropro® - MIC is a system Intended for Antimicrobial Susceptibility Testing and reporting of most pathogens involved in UTI, GI, GT, ENT, CNS, Blood infections etc., in five to eight hours.

Summary

An important task for the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used conventional methods include the disk diffusion method. Later generation testing methods include broth microdilution which include the use of rapid and sensitive instruments.
In general, current testing methods provide accurate detection of antimicrobial resistance. Use of instrumentation can standardize the reading of end points and often produce susceptibility test results in a shorter period. Sensitive optical detection systems allow detection of even subtle changes in bacterial growth leading to faster detection of end points.

Principle

A breakpoint is a chosen concentration (mg/L) of an antibiotic which defines whether a species of bacteria is susceptible or resistant to the antibiotic, which is the criteria for **Micropro® - AST**.

Micropro® - MIC is to determine the minimum inhibitory concentration of antibiotic against the pathogen within five to eight hours.
MIC i.e. Minimum Inhibitory Concentration, is the lowest concentration of an antibiotic required to inhibit the growth of an organism. To determine MIC, concerned pathogen / culture suspension is added to the microwells containing varying concentrations of the antibiotic. The concentration of antibiotic is doubled in each successive microwell and the MIC is found by identifying the first well in which there is no visible colony after an incubation period.
If the MIC is less than or equal to the susceptibility breakpoint the bacteria is considered susceptible to the antibiotic. If the MIC is greater than this value the bacteria is considered intermediate or resistant to the antibiotic.

Working Principle

The **Micropro® - MIC** system is based on three basic steps:

a. Inoculum preparation in Mueller Hinton Broth-CA.
b. Selecting the required **Micropro® Test Panel Kit** and loading the inoculum.
c. Detection of Susceptibility based on growth measured by Turbidimetry Analyzer.

Storage and Stability

a. Store the **Micropro® Test Panel Kit - GN1/ GN3/ GP1**: as mentioned on respective carton / bottle packaging.
b. Avoid exposure to light.
c. The shelf life of **Micropro® Test Panel Kit - GN1/ GN3/ GP1** is as per expiry date mentioned on respective carton / bottle packaging.

Type of specimen

Clinical samples.

Material Required but not provided

Bacteriological Incubator at 35°C-37°C, Marker Pens, Tissue Paper, 70% IPA, Bactericidal hand-rub, gloves and masks.

Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Test Procedure

The user is requested to familiarize with the working of the **Micropro® - MIC** Analyzer before embarking on the Test Procedure. (Refer the **Micropro® - MIC** Analyzer user manual under settings option in **Micropro®- ASTRA** Software User Interface).

A) Culture Selection and Preparation:

1. For all sample types perform Identification and categorize the cultures as Gram positive or Gram negative. If Identification not done then just perform Gram staining.
2. For Gram positive samples select from **Micropro® - MIC Test Panel Kit - GP1** and for Gram negative samples select **Micropro® - MIC Test Panel Kit - GN1 / GN3,** whichever is recommended by the software **Micropro® - ASTRA**, in accordance to CLSI guidelines.

B) Inoculum Preparation:

3. From the recommended **Micropro® - MIC Susceptibility Test Kit**, retrieve the required number of Normal Saline vials and Mueller Hinton Broth-CA vials corresponding to the number of samples to be tested and place them on a flat clean table top.
4. Write Patient IDs / Names in the space indicated on both the vials. Do it for all the samples.
5. Retrieve the required number of Sterile Loops and Sterile Droppers corresponding to the number of samples to be tested.
6. For plate cultures, open the vial of Normal Saline and place it on the flat clean table top. Look for a well isolated single colony in the plate, using a Sterile Loop, pick it and transfer it to the Normal Saline vial. Dissolve the inoculum thoroughly to avoid clumping of the cells. Adjust turbidity of inoculum to match approx. 10^8 cfu/mL using the **Microxpress® McFarland Reader** provided with the Installation pack. Close the Vial and place it separately from uninoculated Normal Saline vials on the table. Do the same for rest of the samples.
7. Retrieve the inoculated Normal Saline and the corresponding Mueller Hinton Broth-CA vials, open and place them on the flat clean table top. Using the Sterile Dropper provided, transfer 3 drops of inoculated Normal Saline to corresponding Mueller Hinton Broth-CA vial. Dissolve the inoculum thoroughly to avoid clumping of the cells. Close the Vials. Discard this inoculated Normal Saline vial. Retain and place the inoculated Mueller Hinton Broth-CA vial separately from uninoculated ones on the table. Do the same for rest of the samples.

C) Loading the Inoculum in Micropro® - MIC Test Panel:

8. Based on the cultures categorized in segment **A** (**Culture Selection and Preparation**), select the appropriate **Micropro® - MIC Susceptibility Test Panels**.
9. Retrieve the required number of **Susceptibility Test Panels** and place them on the table.
10. Retrieve the required number of Gamma Sterile reservoirs corresponding to the number of samples to be tested.
11. Take a pouch of **Susceptibility Test Panel** and tear it open. Place the Panel such that the Test ID code is at your right hand side.
12. Note down the Patient IDs / Names / other details and the corresponding Test-ID Codes in the register. Do it for all the samples.
13. Retrieve the inoculated Mueller Hinton Broth-CA vial and a sterile reservoir. Mix well and open the inoculated Mueller Hinton Broth-CA vial and pour the entire inoculum in a sterile reservoir.
14. Using the Multichannel Stepper Micropipette (8 Channel, 1200 µL) and Sterile Tips in a Tipbox provided, transfer the inoculum from reservoir to all the wells in Susceptibility Test Panel. Use the same set of tips to dispense inoculum twice or thrice for a sample. Discard the tips and the Reservoir. Do the same for all the samples.

D) Initiate test in Micropro® - MIC Analyzer:

15. Refer Help section in **Micropro® - ASTRA** Software User Interface installed in the computer.

E) Incubation of Micropro® - MIC Test Panels:

16. After the test is initiated, put the tray covers on all the Susceptibility Test Panels and place them in a Bacteriological Incubator at 35°C - 37°C.
17. Recommended incubation time is at least 5 hours for Gram negative cultures. For Gram positive cultures extra 2-3 hours of incubation will be required, which will be instructed by the Software. However, it can be incubated further overnight for 16 - 20 hours as per guidelines in CLSI 2019.

F) Fill patient details in Micropro® - ASTRA Software User Interface:

18. In the meantime, fill patient details in **Micropro® - ASTRA** Software User Interface. Refer Help section in **Micropro®-ASTRA** Software User Interface installed in the computer.

G) Check Incubation status in Micropro® - MIC Analyzer / Micropro® - ASTRA Software UI:

19. After the recommended time interval, check whether incubation status is complete. **Micropro® - MIC** Analyzer / **Micropro® - ASTRA** Software User Interface utilizes algorithm and tells the status and further action required. Refer Help section in **Micropro® - ASTRA** Software User Interface installed in the computer.

H) Check result and report:

20. After incubation is over, **Micropro® - ASTRA** Software User Interface performs meticulous calculations to provide MIC values and gives results as Susceptible, Intermediate or Resistant against each antibiotic. Refer Help section in **Micropro® - ASTRA** Software User Interface installed in the computer.
21. Take a print out of the sample result with the printer attached to the computer.

Performance Data

Internal Evaluation

Standard ATCC cultures as recommended by CLSI were used for validation. These cultures were tested on **Micropro® - MIC**. The expected Quality Control Ranges (µg/mL) as depicted in CLSI - 2018 against these reference cultures were compared with the results obtained with **Micropro® - MIC Test Kit**. The results were also compared with standard Kirby–Bauer antibiotic testing method for Sensitive / Intermediate / Resistant results.

Result: Micropro® - MIC showed 100% correlation with both CLSI and Kirby–Bauer antibiotic testing method.

External Evaluation

Conducted in random places, Pan India. A total of 100 specimen were tested simultaneously both on **Micropro® - MIC** and on existing Automated AST Systems.

Result: Micropro® - MIC showed more than 91 % correlation with Automated AST Systems.

Precision Validation

Repeatability and Reproducibility tests were performed with actual samples and control ATCC cultures recommended by CLSI. Same sample was inoculated in five different kits from three different lots.

Result: The result obtained is compared and found to be acceptable within 0.1% discrepancy.

Please refer pack insert for antibiotics list provided in Micropro® - MIC Test panel kits.
Note: The antibiotics list mentioned in pack insert is the complete list present in the panels.
Micropro®-MIC System will generate test report based on recommended antibiotics as per CLSI.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

a. For laboratory use only.
b. Bring all reagents and specimen to room temperature (20°C-30°C) before use.
c. Do not use the kits beyond expiry date.
d. Carefully read the User Manual and package inserts before use.
e. Take Universal Precautions. All human body fluids should be treated as potentially infectious.
f. Always be prepared for any accidental spillage. In case of accidental spillage clean the area thoroughly and wipe with 70% IPA at least three times.
g. It is recommended that basic Personal Protective Equipment like gloves and masks are used at all times.
h. Use a Bactericidal handrub before and after test procedure.
i. Visually examine the Broth, reagents and other components to ensure there is no physical damage, microbial contamination, discoloration, precipitation, evaporation or other signs of deterioration. If any of these is observed, do not use these reagents and contact Service provider immediately.

Cleaning and Decontamination

a) Spills of potentially infectious material should be cleaned up immediately with absorbent tissue paper and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite (10 times dilution of 5% sodium hypochlorite i.e. household bleach) before continuing work.
b) Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially biohazardous waste in a biohazard waste container.

c) Use 70% IPA (Isopropyl alcohol) to decontaminate and clean **Micropro® - MIC** Analyzer, Susceptibility test Panel Tray and Tray cover before and after every test.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. M100S, Performance Standards for Antimicrobial Susceptibility Testing, 29th Edition, CLSI 2019.
2. M07-A9, Vol32, No.2, Methods for Dilution Antimicrobial Susceptibility tests for Bacteria That grow aerobically; approved standard-Nineth Edition, CLSI 2012.
3. Koneman's Color Atlas & Textbook of Diagnostic Microbiology, Lippincott Williams & Wilkins; 6th edition.
4. A Method for Antibiotic Susceptibility Testing: Applicable and Accurate Ramezan Ali Ataee, Ali Mehrabi-Tavana, Seyed Mohammad Javad Hosseini, Khadijeh Moridi, Mahdi Ghorbananli Zadegan, Jundishapur J Microbiol. 2012.
5. Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic Bacteria, Donald R. Stalons and Clyde Thornsberry, Center for Disease Control, 1974.
6. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices, James H. Jorgensen1 and Mary Jane Ferraro, Medical Microbiology, L. Barth Reller and Melvin P. Weinstein, Section Editors, Departments of Pathology and Medicine, Massachusetts General Hospital and Harvard Medical School, Boston.
7. Direct Antimicrobial Susceptibility Testing for Acute Urinary Tract Infections in Women, James R. Johnson, Felice
   S. Tiu and Walter E. Stamm, Journal of Clinical Microbiology, Sept. 1995, p. 2316–2323.
8. Determination of minimum inhibitory concentrations, Jennifer M. Andrews, Journal of Antimicrobial Chemotherapy (2001) 48, Suppl. S1, 5-16.
9. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamawa state, Nigeria, El-Mahmood Muhammad Abubakar, Journal of Clinical Medicine and Research Vol. 1(1) pp. 001-008, October, 2009.
10. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland, Katarzyna Hryniewicza, Katarzyna Szczypab, et.al. Journal of Antimicrobial Chemotherapy (2001) 47, 773–780.
11. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

No.	System Components	Description	Qty.	Cat. No.
Micropro® - MIC Installation Pack			**1 Pack**	209131290001
1	**Micropro® - MIC** Analyzer	Analyzer for reading MIC Test panels	1 Unit	
2	Microxpress® McFarland Reader	McFarland Reader for inoculum preparation	1 Nos.	
3	Test Panel Tray Cover	Tray cover to place over the test panels while testing	10 Nos.	
4	Multichannel Stepper Micropipette (8 channel, fixed 1200 µL)	Micropipette for dispensing inoculum into test panels	1 No.	
5	Gamma Sterile Microtips (1200 µL)	Gamma sterile tip	96 Nos.	
6	Gamma sterile tip box	Gamma sterile tip box for 1200 µL tips	1 No.	
7	Analyzer accessories	User manual, power cable, RS-232 cable, fuse, lamp, etc.	1 No.	
Reagent Packs				
1	**Micropro® - MIC** GN1 Test Kit (30 Tests)	a) Susceptibility Test Panel b) Normal Saline c) MH Broth - CA d) Gamma Sterile Loop e) Gamma Sterile Dropper f) Gamma Sterile Reservoir g) Gamma Sterile Tips (1200 µL) (8 Nos.)	30 Nos. each	209131300030
2	**Micropro® - MIC** GN3 Test Kit (30 Tests)			209131320030
3	**Micropro® - MIC** GP1 Test Kit (30 Tests)			209131330030

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.