Micropro® - AST

Intended Use
Micropro® - AST is a system intended for Antimicrobial Susceptibility Testing of most pathogens involved in UTI, GI, GT, ENT, CNS, Blood etc., within 10-12 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 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.

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® - AST is to determine the minimum inhibitory concentration of antibiotics against the pathogen within 10 to 12 hours.

Working Principle
The **Micropro® - AST** system is based on three basic steps:
1. Inoculum preparation (McFarland standard 0.5) in Mueller Hinton Broth-CA.
2. Selecting the required **Micropro® Susceptibility Test Panel Kit** and loading the inoculum.
3. Detection of Susceptibility based on growth measured by Turbidimetry Analyzer.

Storage and Stability
Store the **Micropro® Susceptibility Test Panel Kit - UTI/ GN/ GN.1/ GN.2/ GP/ GP.1**: as mentioned on respective carton / bottle packaging.
Avoid exposure to light.
The shelf life of **Micropro® Susceptibility Test Panel Kit - UTI/ GN/ GN.1/ GN.2/ GP/ GP.1** 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 a permissible duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Test Procedure
The user is requested to get familiarize with the working of the **Micropro® - AST** Analyzer before embarking on the Test Procedure. (Refer the **Micropro® - AST** Analyzer user manual and help segment in Micropro® - AST software user interface).

A) Culture Selection and Preparation:
1. For UTI samples, Identification of culture is recommended.
2. For UTI cultures **Micropro® - AST Susceptibility Test Panel Kit-UTI** is recommended.
3. For samples from other body sources, perform Identification and categorize the cultures as Gram positive or

Gram negative. If Identification is not done, then just perform Gram staining.

4. For Gram positive samples select **Micropro® - AST Susceptibility Test Panel Kit-GP** and for Gram-negative samples select **Micropro® - AST Susceptibility Test Panel Kit-GN**.

B) <u>Inoculum Preparation:</u>

5. From the recommended **Micropro® - AST Inoculum Preparation Kit**, retrieve the required number of Normal Saline vials and Mueller Hinton Broth-CA vials correspond to the number of samples to be tested and place them on a flat clean tabletop.
6. Write Patient IDs / Names in the space indicated on both the vials. Do it for all the samples.
7. From **Micropro® - AST Accessories Kit** retrieve the required number of Sterile Loops and Sterile Droppers corresponding to the number of samples to be tested.
8. For plate cultures, open the vial of Normal Saline and place it on the flat clean tabletop. Look for a well isolated colony in the plate, using a Sterile Loop, touch 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 the McFarland Standard 0.5 (approx. 10^8 CFU/mL) 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.
9. Retrieve the inoculated Normal Saline and the corresponding Mueller Hinton Broth-CA vials, open and place them on the flat clean tabletop. Using the Sterile Dropper provided, transfer 2 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) <u>Loading the inoculum in Micropro® - AST Susceptibility test Panel:</u>

10. Based on the cultures categorized in segment 9.A (**Culture Selection and Preparation**), select the appropriate **Micropro® - AST Susceptibility test Panel Kit**.
11. From Micropro® - AST Susceptibility Test Panel Kit, retrieve the required number of Micropro® - AST Susceptibility Test Panels and place them on the table.
12. **From Micropro® - AST Accessories Kit** retrieve the required number of sterile reservoirs corresponding to the number of samples to be tested.
13. Retrieve a Susceptibility Test Panel Tray and place it such that the letters A to H showing the rows in the tray are positioned on your left-hand side.
14. Take a pouch of **Micropro® - AST Susceptibility Test Panel** and tear it open. Place the Panel strips on the Susceptibility Test Panel Tray such that the Test ID code is at your right-hand side. It is advised to leave 1 or 2 rows empty from the top to avoid contamination and fast drying.
15. Note down the Patient IDs / Names, the corresponding Test-ID Codes and the row positions (for e.g. B, C or B, C, D etc.) in the register. Do it for all the samples.
16. Retrieve the inoculated Mueller Hinton Broth-CA vial and a sterile reservoir. Mix well, then open the inoculated Mueller Hinton Broth-CA vial and pour the entire inoculum into a sterile reservoir.
17. Using the **Multichannel Micropipette (12 Channel, Variable 20-200 µL)** and Sterile Tips provided, transfer the inoculum from reservoir to the corresponding Micropro® - AST Susceptibility Test Panel strips (B, C or D), one by one to all the two or three strips. 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.

Note: It is recommended to use one tray for one sample, as it will avoid accidental cross contamination and ensure easy handling while testing. Leave at least 2 to 3 rows on the top while placing the strips, to avoid environmental contamination and unwanted faster drying.

D) Initiate test in Micropro® - AST Analyzer:

18. Refer Help section in Micropro®- AST Software User Interface installed on the computer.

E) Incubation of Micropro® - AST Susceptibility Test Panels:

19. After the test is initiated, put the tray covers on Micropro®- AST Susceptibility Test Panel/Trays and place them in a Bacteriological Incubator at 35°C-37°C.

20. Recommended incubation time is at least 10-12 hours for Gram-negative and UTI cultures. For Gram Positive cultures 12-14 hours of incubation is recommended. If additional incubation of 2-3hours is required, it will be instructed by the Software. However, it can be incubated further overnight for 16-20 hours as per CLSI guidelines.

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

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

G) Check Incubation status in Micropro® - AST Analyzer/Software User Interface:

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

H) Check Result and Report:

23. After incubation is over, Micropro® - AST Software User Interface calculates the results as Susceptible, Intermediate or Resistant against each antibiotic and recommends antibiotics with priority as well. Refer Help section in Micropro® - AST Software User Interface installed in the computer. Take a printout 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 simultaneously both on Micropro® - AST and on standard Kirby–Bauer antibiotic testing using Mueller Hinton Agar plate. The expected Susceptibility results as depicted by CLSI against these reference cultures were compared with the results obtained with Micropro® - AST Susceptibility test Panel Kit. The results were also compared with standard Kirby–Bauer antibiotic testing using Mueller Hinton Agar plate.
Result: Micropro® - AST showed 100% correlation with both CLSI and Kirby–Bauer antibiotic testing method.

External Evaluation

Conducted in Goa Medical college, Bambolim Goa. A total of 100 specimens were tested simultaneously both on Micropro® - AST and on standard Kirby–Bauer antibiotic testing using Mueller Hinton Agar plate.
Result: Micropro® - AST showed 91.67 % correlation with Kirby–Bauer antibiotic testing method.

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.

Antibiotics list in Micropro® - AST Susceptibility test Panel Kits:

A. Micropro® - AST Susceptibility test Panel Kit -UTI

Ampicillin
Amikacin
Aztreonam
Amoxicillin-Clavulanic Acid
Ampicillin-Sulbactam
Ciprofloxacin
Ceftriaxone
Cefazolin
Cefepime
Ceftazidime
Fosfomycin
Gentamicin
Levofloxacin
Meropenem
Nitrofurantoin
Norfloxacin
Nalidixic acid
Piperacillin-Tazobactam
Tobramycin
Tetracycline
Trimethoprim- Sulfamethoxazole
Ticarcillin- Clavulanic acid

B. Micropro® - AST Susceptibility test Panel Kit -GN, GN.1 and GN.2

Ampicillin
Amikacin
Aztreonam
Azithromycin
Amoxicillin-Clavulanic Acid
Ampicillin-Sulbactam
Ciprofloxacin
Ceftriaxone
Cefazolin
Cefepime
Ceftazidime
Ceftazidime-Avibactam
Cefoxitin
Colistin
Chloramphenicol
Cefixime
Cefoperazone-Sulbactam
Cefotaxime
Cefuroxime
Doxycycline
Doripenem
Fosfomycin
Gentamicin
Gatifloxacin
Imipenem
Levofloxacin
Meropenem
Minocycline
Nitrofurantoin
Norfloxacin
Nalidixic acid
Netilmicin
Ofloxacin
Piperacillin
Piperacillin-Tazobactam
Tobramycin
Tetracycline
Trimethoprim-Sulfamethoxazole
Ticarcillin-Clavulanic acid

C. Micropro® - AST Susceptibility test Panel Kit -GP and GP.1

Ampicillin
Amikacin
Azithromycin
Ciprofloxacin
Cefepime
Cefoxitin
Chloramphenicol
Cefotaxime
Cefuroxime
Clarithromycin
Clindamycin
Doxycycline
Erythromycin
Fosfomycin
Gentamicin
Gatifloxacin
Levofloxacin
Linezolid
Minocycline
Moxifloxacin
Nitrofurantoin
Norfloxacin
Netilmicin
Oxacillin
Ofloxacin
Penicillin
Rifampin
Tetracycline
Trimethoprim-Sulfamethoxazole
Teicoplanin
Vancomycin

Note: The antibiotics list mentioned here is the complete list for UTI, GN and GP organisms. Micropro®- AST 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 specimens 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 of as potentially biohazardous waste in a biohazard waste container.
c) Use 70% IPA (Isopropyl alcohol) to decontaminate and clean Micropro® - AST 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 for use and sale for any other purpose.

Reference

1. M100, Performance Standards for Antimicrobial Susceptibility Testing, 35th Edition, CLSI 2025.
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, L. Williams & Wilkins; 6th edition.
4. A Method for Antibiotic Susceptibility Testing: Applicable and Accurate Ramezan Ali Ataee, Ali MehrabiTavana, 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 2Pathology and 3Medicine, 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.	Component	Description	Qty.	Cat. No.
Micropro®- AST Installation Pack			1 Pack	209131180001
1	Micropro®-AST Analyzer	Analyzer for Reading AST microplates	1 Unit	
2	Microxpress® McFarland Reader	McFarland Reader for inoculum preparation	1 Unit	
3	Susceptibility Test Panel Tray with Tray cover	Tray to place susceptibility test panels while testing	10 Nos.	
4	Multichannel Micropipette (12 Channel, Variable 20- 200uL)	Micropipette for dispensing inoculum into susceptibility test panels	1 No.	
5	Gamma Sterile Microtips(200uL)	Gamma Sterile tips (200 µL)	12 × 20 Nos.	
6	Gamma SterileTipbox(200uL)	Gamma Sterile tipbox	1 No.	
7	Analyzer accessories	User Manual, Power cable, rs232 cable, fuses. (rs232 port or serial converter if required).	1 No.	
Reagent Packs				
1	Micropro®- AST Susceptibility Test Panel Kit- UTI (20 Tests) a) Micropro®- AST Susceptibility test Panels-UTI	Two Susceptibility Panels with 22 Antibiotics	20 Nos.	209131190020
2	Micropro®- AST Susceptibility Test Panel Kit- GN (20 Tests) a) Micropro®- AST Susceptibility test Panels-GN	Three Susceptibility Panels with 31 Antibiotics	20 Nos.	209131200020
3	Micropro®- AST Susceptibility Test Panel Kit- GN.1 (20 Tests) a) Micropro® -AST Susceptibility test Panels-GN.1	Three Susceptibility Panels with 26 Antibiotics	20 Nos.	209131210020
4	Micropro®- AST Susceptibility Test Panel Kit- GN.2 (20 Tests) a) Micropro®- AST Susceptibility test Panels-GN.2	Three Susceptibility Panels with 33 Antibiotics	20 Nos.	209131220020
3	Micropro®- AST Susceptibility Test Panel Kit- GP (20 Tests) a) Micropro®- AST Susceptibility test Panels-GP	Three Susceptibility Panels with 26 Antibiotics	20 Nos.	209131230020

4	Micropro®- AST Susceptibility Test Panel Kit- GP.1 (20 Tests) a) Micropro®- AST Susceptibility test Panels-GP.1	Three Susceptibility panels with 29 Antibiotics	20 Nos.	209131240020
5	Micropro®- AST Inoculum Preparation Kit (20 Tests) Normal Saline MH Broth-Cation Adjusted	For inoculum Preparation Ready to use Broth for culture growth	24 Nos. 24 Nos.	209131250020
6	Micropro®- AST Accessories Kit (20 Tests) a) Sterile Loop b) Sterile Dropper c) Sterile Reservoir	Gamma Sterile Loops Gamma Sterile Dropper Gamma Sterile Reservoir	20 Nos. 20 Nos. 20 Nos.	209131260020