Micropro® - Identification System

Intended use

The identification system is a miniaturized identification method employing modified conventional and chromogenic substrates. It is intended for the identification of aerobic Gram-negative bacteria from the family Enterobacteriaceae, some frequently isolated fermenting and non-fermenting Gram-negative bacteria and Gram-positive bacteria.

Summary

Micro methods for the biochemical identification of microorganisms were reported as early as 1918. The interest in miniaturized identification systems led to the introduction of several commercial systems in the late 1960s, and they provided advantages in requiring little storage space, extended shelf life, standardized quality control and ease of use. Many of the tests used in the Micropro® - Identification system are modifications of classical methods. These include tests for Fermentation, Oxidation, degradation, and hydrolysis of various substrates. In addition, there are chromogen linked substrates to detect enzymes that microbes use to metabolize various substrates.
Micropro® - Identification system comprised of (i) Micropro® - ID panel (ii) Micropro® - ID Inoculum broth (iii) Micropro® - ID Sterile Paraffin oil. The Panel contains 23 Dehydrated Substrates. The inoculum is prepared with the inoculum broth and is used to fill all the wells. The test inoculum rehydrates the dried substrates and initiates test reactions.
Following an incubation period, the wells are examined for color changes resulting from metabolic activities of the microorganisms. Biochemical and enzymatic reaction patterns for the 23 substrates for a wide variety of organisms are stored in the Micropro® - Identification system database.

Principle

The Micropro® - Identification panels contain 23 dried biochemical and enzymatic substrates. A bacterial suspension in the inoculum broth is used for rehydration of the substrates. The tests used in the system are based on microbial utilization and degradation of specific substrates detected by indicator systems. Chromogenic substrates upon hydrolysis produce color changes. In addition, there are tests that detect the ability of an organism to hydrolyze, degrade, reduce, or otherwise utilize a substrate in the Micropro® - Identification Systems.

Storage and Stability

a) Store the Micropro ® - Identification Kit – GN-1/GP-1: as mentioned on respective carton / bottle packaging. Micropro® - Identification panels are individually packaged and must be stored unopened in a refrigerator at 2 – 8°C. **DO NOT FREEZE**. Visually inspect the package for holes or cracks in the foil package. Do not use it if the packaging appears to be damaged. Panels in the original packaging, if stored as recommended, will retain expected reactivity until the date of expiration.
   For Inoculum broth Visually inspect the tubes for cracks, leaks, etc. Do not use it if there appears to be a leak, vial or cap damage or visual evidence of contamination (i.e., haziness, turbidity). Store vial at 2 – 8°C. Expiration dating is shown on the vial label.
   On receipt, store the Micropro® - Identification kit at 2 – 8°C. Once opened, only the panels and inoculum broth need to be stored at 2 – 8°C. The remaining components of the kit may be stored at 10 – 30°C. If the kit or any of the components are stored refrigerated, each should be brought to room temperature prior to use.
b) The shelf life of Micropro® - Identification Kit - GN-1/GP-1 is as per expiry date mentioned on respective carton / bottle packaging.

Material Required but Not Provided with the Kit

Bacteriological Incubator at 35°C - 37°C non-CO_2 (40 – 60% humidity), Marker Pens, Tissue Paper, 70% IPA, Bactericidal Handrub, Gloves and Masks, Pipette (100µL), aluminum foil.

Precautions

(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 the test procedure.
(i) Visually examine the reagents and other components to ensure there is no physical damage, microbial contamination, or other signs of deterioration. If any of these is observed, do not use these reagents and contact the 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 test Panel Tray and Tray cover before and after every test.

Specimen collection and processing

Micropro® - Identification Systems are not to be used directly with clinical specimens. Use bacterial isolates from media such as Tryptone Soy Agar with 5% Sheep Blood or Columbia Agar with 5% Sheep Blood (Columbia Blood Agar base). Use of selective media such as Phenylethyl Alcohol Agar with 5% Sheep Blood (PEA) or Columbia CNA Agar with 5% Sheep Blood (CNA) is also acceptable. Media containing esculin should not be used. The test isolate must be a pure culture, not more than 18 – 24 h old for most genera; for some slow growing organisms up to 48 h may be acceptable. The incubator used should be humidified to prevent evaporation of fluid from the wells during incubation. The recommended humidity level is 40 – 60%. The usefulness of Micropro® - Identification Systems or any other diagnostic procedure performed on clinical specimens is directly influenced by the quality of the specimens themselves.

Test Procedure

Culture Selection and Preparation:

1) Use isolates from nutrient agar plate or blood agar plate. Also isolates from MacConkey Agar plate are acceptable. The test isolate must be **pure culture** and not more than 24 h old.
2) For all samples perform Gram Screening (Rapid Gram Screening Kit, Cat no.203180350100) or Gram Staining and categorize the cultures as Gram Positive or Gram Negative.
3) For Gram Negative (GN) samples select Micropro® - Identification Kit – GN-1 and For Gram Positive (GP) samples select Micropro® - Identification Kit – GP-1.

Inoculum Preparation:

4) From the Micropro® - Inoculum Preparation Kit, retrieve the required number of Inoculum Broth vials corresponding to the number of samples to be tested and place them on a flat clean tabletop.
5) Write Patient IDs / Names in the space indicated on the respective vials for all the samples.
6) Open a vial of Inoculum Broth and place it on the flat clean tabletop. Look for a well isolated single colony in the plate, pick it up using a Sterile Loop and transfer it to the Inoculum Broth Vial. Dissolve the inoculum thoroughly to avoid clumping of the cells (Vortex for approximately 10-15 sec). Adjust the turbidity equivalent to **McFarland No. 2** standard using Microxpress® **McFarland Reader V.2** provided with the Installation pack. Do the same for the rest of the samples.

If the inoculum suspension concentration is more than the recommended McFarland standard, one of the following steps is recommended:

1. Use a fresh tube of inoculum broth to prepare a new inoculum suspension equivalent to a **McFarland No. 2** standard.
2. If additional colonies are unavailable for preparation of a new inoculum suspension, aseptically, dilute the inoculum by adding the minimum required volume of inoculum broth to bring down the turbidity equivalent to **McFarland No.2.**

Loading the Inoculum in MICROPRO®- Identification Panel:

7) Retrieve the required number of Test Panel pouches and place them on the table.
8) Remove Panel from the pouch and discard the desiccant. Once removed from the pouch, Panels should be used within 1 hr. Do not use the panel if there is no desiccant in the pouch. Place the Panel strips on the Test Panel Tray.
9) Note down the Patient IDs / Names / other details in the register for all the samples.
10) Retrieve the inoculated inoculum broth vial and a sterile reservoir. Mix well and open the inoculated inoculum broth vial and pour the entire inoculum broth in a sterile reservoir.
11) Using the **Multichannel Micropipette (12 Channel, Variable 20-200uL)** load 100µL inoculum in each of the 24 wells using Gamma sterile Tips provided.
12) Add 50 µL sterile Paraffin oil in **well A10 /A11 /A12 for GN-1** and **B10 /B11 for GP-1 (Immediately).** Do the same for all the samples.

Initiate test in Micropro® - MIC Analyzer:

13) Refer Help section in Micropro® - ASTRA Software User Interface installed in the computer.

Incubation of MICROPRO®- Identification Panels:

14) Cover the Test Panel Tray with tray cover and aluminum foil. Place them at 35°C -37°C in a non-CO_2 incubator with 40- 60% humidity. Trays should not be stacked more than two during incubation. The incubator door should not be opened repeatedly during incubation period.
15) Recommended incubation time is at least 12 hours. (Can be incubated overnight).
16) Panels should be read within 30 mins after being removed from incubator.

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

17) 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.

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

18) After the recommended time interval, check whether incubation status is complete. Micropro® - MIC Analyzer / Micropro® - ASTRA Software User Interface utilizes algorithms and tells the status and further action required.

Check result and report:

19) After incubation is over, Micropro® - ASTRA Software User Interface performs meticulous calculations to provide the identification result.
20) Take a printout of the sample result with the printer attached to the computer.

Performance Data

Internal Evaluation

Standard ATCC cultures were used for validation. These cultures were tested simultaneously both on Micropro® - ID and on standard Biochemical testing.

Precision Validation

Repeatability and Reproducibility tests were performed with actual samples and control ATCC cultures. Same samples were inoculated in five different kits from three different lots.
Result: The result obtained is compared and found to be acceptable within 0.1% discrepancy.

User Quality Control:

Quality control testing is recommended for each lot of panels as follows –
We recommend using control strains of known cultures to Verify that the used methodology, tests development and color reaction are correct. It is recommended to use Control strains always when a new batch of the kit is used. Fresh isolates of the control strains must be used to check the functionality of the kits.

LIMITATIONS OF THE PROCEDURE

The Micropro® Identification System is designed for the taxa provided. Taxa other than those listed in Table 1 A & B are not intended for use in this system.

The Micropro® ID database was developed with Microxpress® brand media. Reactivity of some substrates in miniaturized identification systems may be dependent upon the source media used in inoculum preparations. We recommend the use of the following media for use with the Micropro® ID System: Soyabean Casein Digest Agar (TSA) or Columbia Blood Agar. Media containing esculin should not be used.

Micropro® Identification Systems use a modified microenvironment; therefore, expected values for its individual tests may differ from information previously established with conventional test reactions. The accuracy of the Micropro® ID System is based on statistical use of specially designed tests and an exclusive database.

While Micropro® ID System aids in microbial differentiation, it should be recognized that minor variations may exist in strains within species. Use of panels and interpretation of results requires a competent microbiologist. The final identification of the isolate should take into consideration the source of the specimen, aerotolerance, cell morphology, colonial characteristics on various media as well as metabolic end products as determined by gas-liquid chromatography, when warranted.

The incubator where panels are placed should be humidified to prevent evaporation of inoculum fluid from the wells during incubation. The recommended humidity level is 40 – 60%.

If the Micropro® ID test profile yields a “No identification” result and culture purity has been confirmed, then it is likely that
(i) the test isolate is producing atypical Micropro® ID reactions (which may also be caused by procedural errors),
(ii) the test species is not part of the intended taxa or
(iii) the system is unable to identify the test isolate with the required level of confidence. Conventional test methods are recommended when user error has been ruled out.

Tabel 1: **A.- GN-1**

Enterobacter aerogenes
Escherichia coli
Klebsiella pneumoniae
Proteus mirabilis
Proteus vulgaris
Salmonella spp.
Shigella flexneri
Shigella boydii
Citrobacter freundii
Pseudomonas aeruginosa

B- **GP-1**

Enterococcus. faecalis
Bacillus. subtilis
Staphylococcus. aureus
Staphylococcus. epidermidis
Listeria. monocytogenes

System components:

No.	System Components	Description	Qty.	Cat. No.
Micropro® - ID Installation Pack			1 Pack	**209132310001**
1.	**Micropro® - Microbial Susceptibility and Identification System Analyzer**	Analyzer for reading ID Test panels	1 Unit	
2.	**Microxpress® McFarland Reader V.2**	McFarland Reader V.2 for inoculum preparation	1 Unit	
3.	**Micropro ® -ID Test Panel Tray with Tray Cover**	Tray to place ID Panels while testing	2 nos.	
4.	**Multichannel Micropipette (12 Channel, Variable 20-200uL)**	Micropipette for dispensing inoculum into identification test panels	1 No.	
5.	**Gamma Sterile Microtips (200 µL)**	Gamma Sterile Filter-tips (200 µL) in a tipbox	12 × 22 Nos.	
6.	**Gamma Sterile Tipbox (200 µl)**	Gamma sterile tipbox	1 No.	
7.	**Multichannel Stepper Micropipette (8 Channel, 1200 µL)**	Micropipette for dispensing inoculum	1 No.	
8.	**Gamma Sterile Micro Tips (1200 µl)**	Gamma sterile tips	8 x 30 Nos.	
9.	**Gamma Sterile Tip Box (1200 µL)**	Gamma sterile tip box.	1 No.	
10.	**Analyzer accessories**	User Manual, Power cable, RS-232 cable, fuse, lamp, mouse.	1 No.	

Reagent Pack				
1.	**Micropro®- ID GN 1 Test Kit (20 Tests)**	Precoated wells with substrates for Identification of pathogens	1 Kit	**209132210020**
2.	**Micropro® -ID GP-1 Kit (20 Test)**	Precoated wells with substrates for Identification of pathogens	1 Kit	**209132220020**
3.	**Micropro®- ID Inoculum Preparation Kit (20 Tests)** **a) Buffer**	For inoculum preparation	20 x 3 mL	**209132230020**
	b) Micropro®- ID Sterile paraffin Oil	For testing identification tests panels.	1 X 3 mL	
4.	**Micropro®- ID Reagent Accessories (20 Tests)** **a) Gamma Sterile Tips (200 µl)**	Gamma Sterile tips without tipbox.	12 x 22 Nos.	
	b) Gamma Sterile Loops	Gamma Sterile for inoculum preparation.	1 x 20 Nos.	
	c) Micropro®- ID Test Panel Tray with Tray Cover	Tray to place identification test panels while testing and cover for protection against dust.	2 Nos.	**209132240020**
	d) Gamma Sterile Reservoirs	Gamma sterile reservoirs for inoculum preparation.	1 x 20 Nos.	