remel

MICRO-ID®

Microbiological Identification System

INTENDED USE

Remel MICRO-ID® is a self-contained unit providing fifteen biochemical tests for use in qualitative procedures for rapid differentiation of the *Enterobacteriaceae*.

SUMMARY AND EXPLANATION

The family *Enterobacteriaceae* includes many clinically important pathogenic microorganisms. Proper patient care requires the prompt and accurate identification of the infective organism. Identification is typically made by comparing the results of a series of biochemical reactions exhibited by the organism isolated in pure culture with the known reactions of all possible organisms.

Conventional biochemical tests generally require organism growth in complex, unstable media, and the results of these tests are not available for 24 hours or longer. Reagents for many biochemical tests have been applied to filter-paper strips which provide reproducible test results four hours after the primary isolation of microorganisms.[1]

MICRO-ID® is based on the principle that the inoculum contains preformed enzymes at levels detectable in four hours by means of a sensitive indicator system. MICRO-ID® is a self-contained unit with reagent-impregnated paper disks for fifteen biochemical tests useful in the differentiation of the family *Enterobacteriaceae*, and is packed in a sealed foil pack containing a silica gel desiccant. MICRO-ID® has a 95% or greater correlation with identifications by overnight, conventional systems.[2-8]

PRINCIPLE

MICRO-ID® contains filter-paper disks impregnated with reagents which detect the presence of specific enzymes and/or metabolic end-products produced by certain microorganisms. These reagents include a substrate to be acted upon by a bacterial enzyme and a detection system which reacts with the metabolic end-product to yield a readily identifiable color change. Precise quantities of substrate and/or detection reagents are applied to each disk. The MICRO-ID® system consists of a molded, styrene tray containing fifteen reaction chambers and a hinged cover. The first five reaction chambers contain a substrate disk and a detection disk. The remaining ten reaction chambers each contain a single, combination substrate/detection disk.

In the VP test, glucose and pyruvate are converted to acetyl-methylcarbinol which is oxidized to diacetyl with KOH. This forms a colored complex with arginine and α-naphthol. In the Nitrate test, nitrate is reduced to nitrite which diazotizes sulfanilic acid. This couples with an α-naphthylamine derivative to form a colored complex. In the PD test, phenylalanine is deaminated to phenylpyruvate which produces a color with ferric ions. In the H_2S test, sodium thiosulfate is reduced to H_2S which reacts with lead acetate to produce black lead sulfide. In the Indole test, tryptophane is metabolized to indole which forms a colored complex with ρ-dimethylaminobenzaldehyde. In the Ornithine test, ornithine is decarboxylated to putrescine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple. Likewise, in the Lysine test, lysine is decarboxylated to cadaverine which raises the pH of the suspension and changes the color of the indicator, bromcresol purple. In the Malonate test, malonate is oxidized to alkaline products which change the color of the indicator, bromthymol blue. In the Urea test, urea is hydrolyzed to ammonia and carbon dioxide with a resulting increase in pH changing the color of the indicator, cresol red. In the Esculin test, esculin is hydrolyzed to glucose and esculetin. Esculetin reacts with ferric ions to give a black color. In the ONPG test, o-Nitrophenyl-β-D-galactopyranoside is hydrolyzed by β-galactosidase to yellow o-nitrophenol and β-D-galactose. Acid metabolic products produced from the fermentation of Arabinose, Adonitol, Inositol, and Sorbitol change the color of the indicator, bromcresol purple.

REAGENTS (CLASSICAL FORMULA)*

TEST	ACTIVE INGREDIENTS PER TEST	
VP - Voges-Proskauer (Acetoin Production)	Glucose	2.5 mg
	Sodium Pyruvate	0.6 mg
	Arginine	0.16 mg
	α-Naphthol Derivative	0.2 mg
N - Nitrate Reductase	Potassium Nitrate	0.07 mg
	Sulfanilic Acid	0.04 mg
	α-Naphthylamine Derivative	0.03 mg
PD - Phenylalanine Deaminase	Phenylalanine	0.55 mg
	Ferric Chloride	0.16 mg
H_2S - Hydrogen Sulfide	Sodium Thiosulfate	0.31 mg
	Lead Acetate	0.25 mg
I - Indole	Tryptophane	0.04 mg
	ρ-Dimethylaminobenzaldehyde	0.17 mg
OD - Ornithine Decarboxylase	Ornithine	0.9 mg
	Bromcresol Purple	0.004 mg
LD - Lysine Decarboxylase	Lysine	1.5 mg
	Bromcresol Purple	0.004 mg
M - Malonate Utilization	Sodium Malonate	0.7 mg
	Bromthymol Blue	0.009 mg
U - Urease	Urea	1.2 mg
	Cresol Red	0.005 mg
E - Esculin Hydrolysis	Esculin	0.06 mg
	Ferric Ammonium Citrate	0.25 mg
ONPG - β-Galactosidase	ONPG	0.46 mg
ARAB - Arabinose Fermentation	Arabinose	2.4 mg
	Bromcresol Purple	0.01 mg
ADON - Adonitol Fermentation	Adonitol	2.4 mg
	Bromcresol Purple	0.01 mg
INOS - Inositol Fermentation	Inositol	2.4 mg
	Bromcresol Purple	0.01 mg
SORB - Sorbitol Fermentation	Sorbitol	2.4 mg
	Bromcresol Purple	0.01 mg

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used.

PRODUCT DETERIORATION

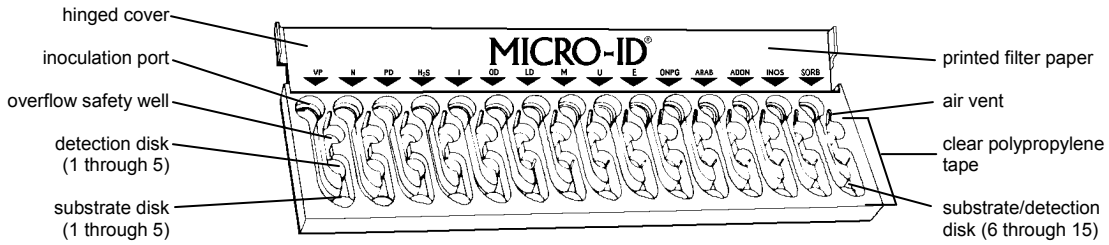
This product should not be used if (1) the foil packet has been damaged or opened prior to use, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[9]

MATERIALS SUPPLIED

1. MICRO-ID® units (10) 2. Encoding forms (12)



MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Physiological saline, (7) Test tubes (16 x 100 mm or larger), (8) Pipettes, (9) Oxidase test reagent (REF R38191 or R21540), (10) MICRO-ID® Identification Manual (REF R38146), (11) Gram stain reagents, (12) McFarland 0.5, 1.0, and 2.0 turbidity standards or equivalents (REF R20410, R20411, R20412), (13) 20% Potassium Hydroxide (KOH) (REF R21231), (14) Syringe, needle, (15) Centrifuge.

PROCEDURE

Inoculum Preparation:

A. Preparation of inoculum from isolated colonies.

1. Select several morphologically identical, well-isolated colonies from the primary isolation plate or other agar medium. Specimens must be properly streaked for isolation of well-defined colonies.[8] Eosin Methylene Blue (EMB), MacConkey, Salmonella-Shigella (SS), Hektoen Enteric, and Blood agars are suitable sources for inoculum. Other media, such as TSI, SIM, LIA, or TSA may also be used. Direct use of broth cultures is not recommended. Use only fresh cultures (18-24 hours old). Cultures older than 30 hours may give false negative test results.
2. Using a portion of the well-isolated colonies, perform a Gram stain and cytochrome oxidase test. Only gram-negative, cytochrome oxidase-negative rods should be tested with the MICRO-ID® system.
3. Thoroughly suspend the remaining colonies in approximately 3.5 ml of physiological saline, in a 16 x 100 mm (or larger) tube (sterile tubes and sterile saline are not required). The number of colonies required will vary according to colony size on the primary plate. The organism suspension should have a clearly visible turbidity equal to at least a 0.5 McFarland turbidity standard or equivalent. Stock culture organisms may be found to exhibit lower levels of enzyme activity than fresh clinical isolates. Accordingly, a denser inoculum, approximately equal to a 2.0 McFarland turbidity standard or equivalent, is recommended for use with stock cultures.

B. Preparation of inoculum from blood culture for presumptive* identification.[7]

1. After the detection of bacterial growth in a blood culture, aseptically remove a small aliquot (approximately 0.5 ml) of broth medium from above the blood cell layer and perform a Gram stain.
2. Aseptically remove approximately 10 ml of medium from above the blood cell layer using a sterile needle and syringe.
3. Centrifuge the 10 ml aliquot for 15 minutes at 1250 x g to obtain a bacterial pellet.
4. Decant and discard the supernatant.
5. Perform a cytochrome oxidase test using a small portion of the bacterial pellet. Only gram-negative cytochrome oxidase-negative bacilli, morphologically consistent with the *Enterobacteriaceae* and appearing to be in pure culture are suitable for testing by the MICRO-ID® procedure.
6. Resuspend the pellet in sufficient physiological saline (at least 3.5 ml) to achieve a turbidity equal to a 0.5-1.0 McFarland standard or equivalent. If a substantially denser inoculum is obtained by this procedure, it is recommended that the inoculum be adjusted in this range with additional saline.
7. Use 3 ml of this suspension for the inoculation of MICRO-ID®. Excess suspension may be used in the inoculation of primary isolation media, growth media, or for direct antimicrobial susceptibility testing if sterile saline and aseptic techniques have been used.

***Note**: Direct identification test results should be considered presumptive until confirmed using the standardized MICRO-ID® technique (i.e., using colonies obtained through routine subculture techniques).

Inoculation, Incubation, and Reading of MICRO-ID®:

1. Open the sealed, moisture-proof, foil package and remove the MICRO-ID® unit. Do not remove the clear plastic tape covering test wells.
2. Record specimen number and other required information on the area provided on the right side of the cover.
3. Open the cover and allow the MICRO-ID® unit to lie flat on the bench.
4. Pipette approximately 0.2 ml of organism suspension into each inoculation well at the top of the MICRO-ID® unit.
5. Close the cover and stand the MICRO-ID® tray upright. (Make sure the organism suspension is in contact with all substrate disks. DO NOT moisten detection disks).
6. Incubate upright for 4 hours at 35-37°C in ambient air (i.e., non-CO_2 incubator).
7. After the four-hour incubation period, place unit flat on bench, open the lid, and add two drops (approximately 0.1 ml) of 20% KOH to the inoculation well of the VP test ONLY. Do not add KOH to any other inoculation well.
8. Close the lid and hold tray upright. Be certain that the KOH flows down into the VP test solution.
9. Rotate the MICRO-ID® unit clockwise about 90 degrees so that the upper disks in the first five wells become wet. Hold the tray upright and tap gently on the laboratory bench to dislodge any suspension trapped under the upper disks. Be certain that each upper disk in reaction chambers 1 through 5 is moistened by this procedure.

10. Read all reactions immediately, except the VP test, as positive or negative according to the color changes listed under INTERPRETATION. Read the color of the upper disk for the first five tests and the color of the organism suspension for the remaining ten tests.

11. Allow color to develop in the VP well for approximately ten minutes, prior to reading.

12. Identification of organisms:
    a. Visual Identification - Identification of the organism is made by comparing the results of the 15 biochemical tests with typical reactions for the family *Enterobacteriaceae*. The MICRO-ID® differentiation checkerboard included in the MICRO-ID® Identification Manual summarizes the biochemical reactions of *Enterobacteriaceae* with the MICRO-ID®. In the procedure, key recognition tests are used to include or exclude a possible identification based on the user's knowledge of microbial taxonomy of the family *Enterobacteriaceae*.

    b. MICRO-ID® Identification Manual - A more complete and rapid identification of members of the family *Enterobacteriaceae* may be made using the MICRO-ID® Identification Manual. This system is based on the simple computation of a five-digit, octal number for a given set of positive and negative test reactions for the 15 biochemical tests. The five-digit, octal number, computed with the aid of the MICRO-ID® Encoding Form, may then be found in the MICRO-ID® Identification Manual which will indicate four possible identification choices and their degree of computed fit to the data. Complete instructions for the calculation and use of the octal numbers are furnished with each MICRO-ID® Identification Manual.

INTERPRETATION

Test	Positive Reaction	Negative Reaction
VP	Pink to Red	Light Yellow
N	Red	Colorless to Light Pink
PD	Green[1]	Light Yellow
H_2S	Brown to Black[2]	White
I	Pink to Red	Light Yellow to Orange
OD	Purple to Red-Purple	Amber to Yellow
LD	Purple to Red-Purple	Amber to Yellow
M	Green to Blue	Yellow
U	Orange to Red-Purple	Yellow
E	Brown to Black	No color change or Beige
ONPG	Light Yellow to Yellow	Colorless
ARAB	Yellow to Amber	Red-Purple to Purple
ADON	Yellow to Amber	Red-Purple to Purple
INOS	Yellow to Amber	Red-Purple to Purple
SORB	Yellow to Amber	Red-Purple to Purple

[1] In the PD test, any green color in the organism suspension also indicates a positive reaction.

[2] A positive H_2S reaction might vary from a thin, dark line at the bottom of the detection disk to the entire disk turning black.

QUALITY CONTROL

All lot numbers of MICRO-ID® have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient/sample results should not be reported.

ORGANISM	VP	N	PD	H_2S	I	OD	LD	M	U	E	ONPG	ARAB	ADON	INOS	SORB
Escherichia coli ATCC® 25922	–	+	–	–	+	+	+	–	–	–	+	+	–	–	±
Proteus vulgaris ATCC® 6380	–	+	+	+	+	–	–	–	+	+	–	–	–	–	–
Klebsiella pneumoniae ATCC® 13882	+	+	–	–	–	–	+	+	±	+	+	+	+	+	+
Acinetobacter baumannii ATCC® 19606	*	–	*	*	*	*	*	*	*	*	*	*	*	*	*

*Not applicable.

LIMITATIONS

1. MICRO-ID® is designed specifically for use with gram-negative rods which are cytochrome oxidase-negative and should only be used to differentiate members of the family *Enterobacteriaceae*. Interpretation of results requires a competent microbiologist who should use judgment based on knowledge and on confirmatory tests, when required, before deciding on the identity of an organism.
2. Serological typing is always required for the final identification of any organism which is biochemically identified as a *Salmonella* or *Shigella*.
3. Supplementary data or additional biochemical tests may be required for species identification of occasional isolates which do not show clearly differentiating patterns.
4. The genus *Yersinia* is a part of the family *Enterobacteriaceae*, and three species are recognized human pathogens. *Y. pestis* is a rare isolate and only those laboratories with highly trained personnel and special equipment (laboratory hoods and proper gowns, masks, and gloves) should attempt to work with this highly dangerous organism. In every case where *Y. pestis* is suspected in a specimen or culture, all deliberate care and caution must be fully exercised, and responsible state or federal laboratories must be notified immediately.
5. Proper bacteriological technique is always required when working with pathogenic organisms. However, sterile saline, pipettes or tubes are not required since all MICRO-ID® reactions are normally completed within four hours. Aseptic technique is not required when removing MICRO-ID® trays from the moisture-proof package.

PERFORMANCE CHARACTERISTICS

Performance for this product is indicated in the Differentiation Checkerboard in the MICRO-ID® Identification Manual.

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PACKAGING

REF R38145, MICRO-ID®..... 10 Tests/Kit

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

MICRO-ID® is a registered trademark of Thermo Fisher Scientific and its subsidiaries.
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IFU 38145, Revised March 14, 2011 Printed in U.S.A.

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