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Microbiological Assay

REF **ME0201 /ME0202 / ME0203**

10 tests/20 tests/50 tests

Gram Negative bacteria ID/AST

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		date of manufacture
	do not reuse		

EC REP

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CE IVD

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Intended use

The Gram Negative bacteria ID/AST is intended for the in vitro identification (ID) and in vitro determination of minimal inhibitory concentration (MIC) for Aerobic and facultative anaerobic Gram Negative bacteria.

Summary

Micromethods for the biochemical identification of microorganisms were reported as early as1918[1].Bacterial identification is the process of analyzing unknown bacteria with known bacteria according to their biological characteristics to determine the taxa (family, genus, species or higher taxa) of unknown bacteria. The main methods include biochemical identification and genotype identification, serological identification and proteomics identification, etc.

The primary objective of susceptibility testing in the clinical setting is to predict the outcome of treatment of an infected individual with the chosen antimicrobial agents. Results must be derived in a timely manner with a high degree of accuracy and reproducibility. Antimicrobial agent susceptibility testing (AST) is usually carried out to determine which antimicrobial agent will be most successful in treating a bacterial infection in vivo. Clinical laboratories can choose to determine the susceptibility of bacteria by a number of methods mainly including disk diffusion, dilution (broth microdilution method, broth macrodilution method and agar dilution), serial dilution (E-test) minimum inhibitory concentration (MIC) determination [2,3], of which can be performed manually or with automation.

The MIC of an organism is defined as the minimum amount of antimicrobial agent required to inhibit the growth of the test organism over a specified time interval (which is related to the growth rate of the bacteria). The rapid and accurate determination of the MIC value for an antimicrobial agent/organism combination can significantly improve patient management and prognosis since it enables the prompt administration of appropriate antimicrobial agent therapy.

Measurement Principle

This test consists of two parts: identification and antimicrobial susceptibility test. The bacteria identification test is achieved by detecting the some biochemical reaction indicators, such as carbon source utilization, enzyme activity, and antimicrobial resistance. Then comparing and analyzing the identification results with the database to confirm the final identification results of the bacteria. The antimicrobial susceptibility test is based on broth microdilution method and redox method [4,5,6]. The AST Plate is coated with varying concentrations of antimicrobial agents at appropriate well locations. The AST Broth with the targeted organism (density of 2.5-3.0 McFarland) and ID Diluent are added with the colorimetric redox indicator (colorimetric oxidation-reduction) to make an inoculum suspension. The suspension and the AST Plate are used into the instrument for inoculation and incubation. After incubation, the minimum inhibitory concentration (MIC) is read according to the changes to the Indicator as well as bacterial turbidity.

Materials provided

1. Reagent pack

Σ	10 tests	20 tests	50 tests
ID/AST Plate	10 plates	20 plates	50 plates
AST Broth	16.0 mL*10	16.0 mL*20	16.0 mL*50
ID Diluent	8.0mL*10	8.0mL*20	8.0mL*50
Indicator Solution	3.0 mL*1	3.0 mL*1	3.0 mL*1

Note: The volume of the AST Broth, ID Diluent and Indicator Solution indicated in above table is the minimum dispensing volume.

- ID/AST Plate

The AST Plate is coated with identification substrates and varying concentrations of antimicrobial agents. The distribution of antimicrobial agents on AST Plate is shown on Table 1.

- AST Broth

The CAMHB broth is used to enrich for targeted organism for detection.

- ID Diluent

The ID Diluent contains Nacl.

- Indicator Solution

The Indicator Solution contains the colorimetric redox.

Table 1: Distribution of antimicrobial agents

Item	1	2	3	4	5	6	7	8	9	10	11	12
A	ADH	LDC	ODC	ODEC	CIT	SUCT	MNT	IMLTa	O129/R	BGUR	BXYL	URE
B	AGAL	BGAL	AGLU	BGLU	RHA	MDG	RAF	INO	LARA	NEG	NAGA	BNAG
C	ADO	dCEL	dGLU	dMAL	dMAN	dMNE	dSOR	TRE	SAC	PLE	dTAG	ESC
D	CON	LAC	SAL	XYL	GLY	NAG	LAP	PYR	PAP	GlyA	3OMG	dMEL
E	FEP 1	FEP 2	FEP 3	FEP 4	FEP 5	CZO 1	CZO 2	CZO 3	CZO 4	CZO 5	TGC 1	TGC 2
F	AMP 1	AMP 2	AMP 3	CRO 1	CRO 2	CRO 3	CRO 4	CRO 5	CRO 6	MNO 1	MNO2	MNO 3
G	CAZ 1	CAZ 2	CAZ 3	CAZ 4	CSL 1	CSL 2	CSL 3	AMK 1	AMK 2	AMK 3	CXM 1	CXM 2
H	TZP 1	TZP 2	TZP 3	TZP 4	SAM 1	SAM 2	SAM 3	TOB 1	TOB 2	TOB 3	CXM 3	CXM 4
I	LVX 1	LVX 2	LVX 3	LVX 4	LVX 5	LVX 6	LVX 7	POL 1	POL 2	POL 3	SXT 1	SXT 2
J	MEM 1	MEM 2	MEM 3	MEM 4	MEM 5	IPM 1	IPM 2	IPM 3	IPM 4	IPM 5	NIT 1	NIT 2

Abbreviation: ADH-Arginine dihydrolase, LDC-Lysine Decarboxylase, ODC-Ornithine decarboxylase, ODEC-Decarboxylase negative control, CIT-Sodium citrate, SUCT-Succinate, MNT-Malonate, IMLTa-Malate, O129/R-O129 resistance, BGUR-β-glucuronidase, BXYL-β-xylosidase, URE- Urease, AGAL- alpha-galactosidase, BGAL-β-galactosidase, AGLU-alpha-glucosidase, BGLU-β-glucosidase, RHA- L-rhamnose monohydrate, MDG-α-methyl-d-glucopyranoside, RAF-Raffinose, INO-Inositol, LARA-L-arabinol, NEG-Negative Control, NAGA- N-acetyl β-galactosaminidase, BNAG-β-N-acetylglucosidase, ADO-Side Calendula Alcohol, dCEL- D-cellobiose, dGLU-D-glucose, dMAL- D-maltose, dMAN- D-mannitol, dMNE- D-Mannose, dSOR -D-Sorbitol, TRE- Trehalose, SAC- sucrose, PLE-Palatinose, dTAG- D-tagatose, ESC- Esculin, CON-Positive Control, LAC- lactose, SAL- Salicin, XYL- Xylose, GLY- glycerin, NAG-N-Acetyl Glucosamine, LAP- Leucine aminopeptidase, PYR- Pyrrolidone aminopeptidase, PAP- Proline aminopeptidase, GlyA- Glycine arylaminease, 3OMG-3-O-Methyl Glucopyranose, dMEL- D-Melibiose, FEP- Cefepime, CZO- Cefazolin, TGC- Tigecycline, AMP- Ampicillin, CRO- Ceftriaxone, MNO- Minocyline, CAZ- Ceftazidime, CSL- Cefoperazone/Sulbactam, AMK- Amikacin, CXM- Cefuroxime, LVX-Levofloxacin, POL- Polymyxin B, SXT- Trimethoprim-sulfamethoxazole, MEM- Meropenem, IPM- Imipenem, NIT- Furantoin;

Automated Microbiology System Analyzers on which the kit can be used

- AutoMic-i600

Materials Required but not Provided

1. Automated Microbiology System Analyzers
2. Micropipettor
3. Vibrator
4. Sterile tips or swabs
5. Nephelometer
6. Turbidity tubes
7. Sterile saline

Warnings and Precautions

1. For professional use only. For in vitro diagnostic use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Wear protective clothing and disposable gloves when dealing with strips. Wash hands after operations. Handle the potentially contaminated materials safely according to local requirement.
4. Standard guidelines for the safe handling and disposal of infectious organisms should be observed throughout all procedures.
5. Do not smoke, drink, eat or use cosmetics in the working area.
6. Do not use, if the plate or packaging appears to be damaged or turbidity precipitation appears in the broth.
7. Micropipettor tips cannot be mixed to avoid contamination.
8. Avoid testing in harsh environments (such as those containing 84 disinfectants, sodium hypochlorite, acid, alkaline, or acetaldehyde and other high concentration corrosive gases and dust).
9. The AST Plate cannot be reused.
10. Do not mix or use components from kits with different batch codes.

Storage

1. Store all components at 2-8℃. When stored as directed, all reagents are stable until the expiration date.
2. The AST Plate should be used within 8 hours once opened; the AST Broth and ID Diluent should be used immediately once opened; store the Indicator Solution sealed at 2-8℃ after opened. It cannot be used beyond the expiration date.

Sample

1. Collect and prepare samples in accordance with the local requirement.
2. The test isolate must be a pure culture. The pure bacteria should be used within 24 hours at room temperature or 2-8℃. For the clinically cryopreserved pure bacteria, it is recommended they should be used after activation and subculture.
3. The preparation and testing of bacteria suspension should be completed within 60 minutes.

Measurement Procedure

1. **Prepare the materials**

- Remove the kit from the refrigerated environment and return to room temperature before opening the package.

2. Order tests

For both Identification test and antimicrobial susceptibility test at the same time

1) Add 2mL sterilized saline water into turbidity tube, pick several pure culture isolates, mix it well in the turbidity tube, and prepare 2.5-3.0 McFarland bacterial suspension by using Nephelometer.
2) Add 2mL bacterial suspension into 1 vial ID Diluent.
3) Add one drop of Indicator Solution into the AST Broth.
4) Place this ID Diluent with bacteria, AST Broth with Indicator Solution and the AST plate in the designed position of instrument, 100μL inoculum is transferred into each well and the AST plate is incubated automatically.

Note: please refer to the AutoMic-i600's operation manual.

For Identification test only

1) Add 2mL sterilized saline water into turbidity tube, pick several pure culture isolates, mix it well in the turbidity tube, and prepare 2.5-3.0 McFarland bacterial suspension by using Nephelometer.
2) Add 2mL bacterial suspension into 1 vial ID Diluent and mix well.
3) Place the ID Diluent with bacteria and the AST plate in the designed position of the instrument, 100μL inoculum is transferred into the corresponding well and the AST plate is incubated automatically.

Note: please refer to the AutoMic-i600's operation manual.

For antimicrobial susceptibility test only

1) Prepare 0.5-0.6 McFarland bacterial suspension with sterilized saline.
2) Add 100μL bacterial suspension and one drop of Indicator Solution into 1 vial AST Broth.
3) Place AST Broth with bacterial and Indicator Solution and the AST plate in the instrument, 100μL inoculum is transferred into each well and the AST plate is incubated automatically.

Note: please refer to the AutoMic-i600's operation manual.

3. Read results

The system could report the identification results, MIC results and Interpretive Categorical Results (SIR). Some MIC results may obtains only MIC without SIR when there is no breakpoint explanation.

Interpretation of Results

1. The Automated Microbiology System Analyzers will report its identification credibility while reporting the identification results. The identification credibility refers to the reliability of the identification results of strains. The specific explanation is shown in the following table 2

Table 2 Identification credibility

Identification credibility	Interpretation of Results
Excellent	No atypical test results
Good	Fewer atypical test results

Acceptable	Few atypical test results
Unacceptable	Atypical biotype, does not match any bacterial results in the database

Note: Atypical test results refer to test results that are contrary to most strains of the same species due to pollution or the appearance of rare biotypes.

2. The Automated Microbiology System analyzer can report the MIC and SIR results according to the latest antimicrobial susceptibility interpretation standards issued by CLSI or EUCAST or FDA. For the antimicrobial without antimicrobial susceptibility interpretation standard, the Automated Microbiology System analyzer only reports MIC results; For antimicrobial without antimicrobial susceptibility testing standard, the Automated Microbiology System analyzer only report identification results.
3. Unacceptable results maybe caused by the following reasons: the Gram staining result is incorrect; the strain is not pure; the bacterial suspension does not reach the specified concentration; the strain activity is severely reduced; the strain database does not contain such bacteria.
4. Some bacteria may have similar biochemical spectra, which makes it difficult to distinguish. Additional tests prompted by the system can be used to distinguish.
5. When the antimicrobial susceptibility positive control well shows "-", it means that there is no bacterial growth, the antimicrobial susceptibility result of the strain is invalid, and the test should be repeated.
6. When the MIC result is displayed as "-", it means that the result is abnormal, it is recommended to repeat the test.
7. When the MIC result is normal and the antimicrobial susceptibility interpretation is displayed as "-", it means that the antimicrobial agent has no drug susceptibility interpretation standard.

Limitations of the Procedure

1. A Gram stain test is required for the selection of the appropriate AST Plate types. Accurate AST results may not be made without this test.
2. Use only well-isolated bacterial colonies from one of the recommended primary isolation media. Use of mixed colonies could result in inaccurate AST interpretations.
3. A suspension equivalent of 2.5-3.0 McFarland standard must be met a nephelometer. Use of alternate methods for suspension preparation may cause erroneous AST results.
4. After the addition of the Indicator Solution to the broth tubes, mix by inversion. DO NOT VORTEX. Vortexing may cause air bubbles to form in the broth, which can result in inappropriate filling of the AST Plate wells during inoculation.
5. The results of this test indicate the Interpretive Categorical Results (SIR) of *in vitro* microorganisms. The sensitivity of some microorganisms to certain antimicrobial agents may be inconsistent *in vitro* and *in vivo*.
6. The test results of this product are used for clinical reference, and should not be used as only evidence for clinical diagnosis and treatment. It should be combined the Information such as symptoms, medical history, other laboratory tests, and treatment response.
7. This product does not contain atypical strains or rare strain databases, which may result in incorrect identification of these strains.

Performance Characteristics

1. Identification Accuracy

Test with the control strains in Table 3 with three batches of this assay, the identification results were all consistent with the information of the control strains.

Table 3 Quality Control strains

Control Strains	Strain NO.
Escherichia coli	ATCC25922
Enterobacter cloacae	ATCC13047
Klebsiella oxytoca	ATCC700324
Pseudomonas aeruginosa	ATCC27853
Stenotrophomonas maltophilia	ATCC13637
Aeromonas hydrophila	ATCC35654

2. MIC testing

Escherichia coli (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853) were used as the control strains, MICs results of at least 18 out of 19 antimicrobial agents should meet the following criteria (shown in table 4 below).

Table 4: the MIC requirements range for other 17 antibacterial drugs.

Antimicrobial agents	*Escherichia coli* (ATCC25922) (μg/mL)	*Pseudomonas aeruginosa* (ATCC27853) (μg/mL)
Cefepime	≤2	≤4
Cefazolin	≤4	＞32
Tigecycline	≤1	＞2
Ampicillin	≤8	＞32
Ceftriaxone	≤1	≥8
Minocyline	≤4	≥16
Ceftazidime	≤4	≤4
Cefopera-zone/Sulbactam	≤16/8	≤16/8
Amikacin	≤16	≤16
Cefuroxime	≤8	＞32
Piperacillin-tazobactam	≤16/4	≤16/4
Ampicillin/Sulbactam	≤8/4	＞32/16
Tobramycin	≤4	≤4
Levofloxacin	≤0.12	0.5-4
Polymyxin B	≤2	≤2
Trime-thoprim-sulfamethoxazole	≤2/38	＞4/76
Meropenem	≤1	≤1
Imipenem	≤1	≤4
Nitrofurantoin	≤32	＞64

Determine MIC for 3 batches of Gram Negative bacteria ID/AST, the results were met the requirements.

3. Repeatability

Test the control statin *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853) with 10 plates from 1 batch of Gram

Negative bacteria AST, the MICs results of at least 18 out of 19 antimicrobial agents should have a ≥90% consistency with the MIC requirements range. The results were met the requirements.

Literature References

1. Bronfenbrenner, J., and Schlesigner, M.J. 1918. “A Rapid Method for the Identification of Bacteria Fermenting Carbohydrates,” Am. J. Public Health. 8:922-923.

2. Miae Lee, Hae-Sun Chung. Different antimicrobial susceptibility testing methods to detect ertapenem resistance in enterobacteriaceae: VITEK2, MicroScan, Etest, disk diffusion, and broth microdilution, J Microbiol Methods, 2015, 112(197):87-91.

3. F.C. Tenover, Antibiotic Susceptibility Testing, Encyclopedia of Microbiology (Third Edition), 2009: 67–77.

4. Clinical and Laboratory Standards Institute. 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: approved standard. Document M07-A11, Clinical and Laboratory Standards Institute.

5. Clinical and Laboratory Standards Institute. 2019. Performance standards for antimicrobial susceptibility testing: approved standard. Document M100-S29, Clinical and Laboratory Standards Institute.

6. European Committee on Antimicrobial Susceptibility Testing. 2019. Breakpoint tables for interpretation of MICs and zone diameters, Version 9.0, European Committee on Antimicrobial Susceptibility Testing.