

Microbiological Assay












REF MD0301 / MD0302 / MD0303



10 tests / 20 tests / 50 tests

Gram Positive bacteria AST

The Gram Positive bacteria AST is intended for in vitro determination of antimicrobial susceptibility of non-fastidious Gram Positive bacteria.

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Key to Graphical Symbols Used			
	batch code		use by
	manufacturer		contains sufficient for <n> tests
	<i>in vitro</i> diagnostic medical device		temperature limitation
	catalogue number		consult instructions for use
	authorized representative in the European Community		do not reuse
	date of manufacture		

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Introduction

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 disc diffusion, dilution (broth microdilution method, broth macrodilution method and agar dilution), serial dilution (E-test) minimum inhibitory concentration (MIC) determination ^{1,2}, 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 is based on broth microdilution method and redox method ^{3,4}. The AST Plate is coated with varying concentrations of antimicrobial agents at appropriate well locations. The Broth with the targeted organism (density of 0.5 McFarland) is 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. Organism determination is used in the interpretation of the MIC values of each antimicrobial agent producing Susceptible, Intermediate or Resistant (SIR) result classifications.

Components

1. Reagent pack

	10	20	50
AST Plate	10 plates	20 plates	50 plates
Broth	16.0 mL*10	16.0 mL*20	16.0 mL*50
Indicator Solution	3.0 mL*1	3.0 mL*1	3.0 mL*1

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

● AST Plate

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

● Broth

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

● Indicator Solution

The Indicator Solution contains the colorimetric redox.

2. 1 sheet of result reading card

Automated Microbiology System on which the kit can be used

- AutoMic-i600

Materials Required but not Provided

1. Micropipettor
2. Vibrator
3. Sterile tips or swabs
4. Nephelometer
5. Turbidity tubes
6. Incubator

Table 1: Distribution of antimicrobial agents

RIF 1	RIF 2	RIF 3	RIF 4	RIF 5	RIF 6	RIF 7	RIF 8	RIF 9	ERY 1	ERY 2	ERY 3
OXA 1	OXA 2	OXA 3	OXA 4	OXA 5	OXA 6	OXA 7	OXA 8	OXA 9	ERY 4	ERY 5	ERY 6
FOX 1	FOX 2	FOX 3	FOX 4	NIT 1	NIT 2	NIT 3	NIT 4	SXT 1	SXT 2	SXT 3	SXT 4
AMP 1	AMP 2	AMP 3	AMP 4	AMP 5	AMP 6	CPT 1	CPT 2	CPT 3	CPT 4	CPT 5	CPT 6
PEN 1	PEN 2	PEN 3	PEN 4	PEN 5	PEN 6	PEN 7	PEN 8	GEN 1	GEN 2	GEN 3	GEN 4
DAP 1	DAP 2	DAP 3	DAP 4	DAP 5	DAP 6	TEC 1	TEC 2	TEC 3	TEC 4	TEC 5	TEC 6
ORI 1	ORI 2	ORI 3	ORI 4	VAN 1	VAN 2	VAN 3	VAN 4	VAN 5	VAN 6	VAN 7	ERY/CLI 1
LNZ 1	LNZ 2	LNZ 3	LNZ 4	TGC 1	TGC 2	TGC 3	TGC 4	TGC 5	GEH 1	STH 1	CON
CIP 1	CIP 2	CIP 3	CIP 4	CIP 5	CIP 6	TCY 1	TCY 2	TCY 3	TCY 4	TCY 5	TCY 6
MFX 1	MFX 2	MFX 3	MFX 4	MFX 5	CLI 1	CLI 2	CLI 3	CLI 4	CLI 5	CLI 6	CLI 7

Abbreviation: RIF-Rifampicin, ERY-Erythromycin, OXA-Oxacillin, FOX-Cefoxitin, NIT-Furantoin, SXT-Trimethoprim-Sulfamethoxazole, AMP-Ampicillin, CPT-Ceftaroline, PEN-Penicillin, GEN-Gentamicin, DAP-Daptomycin, TEC-Teicoplanin, ORI-oritavancin, VAN-Vancomycin, ERY/CLI-Erythromycin/Clindamycin, LNZ-Linezolid, TGC-Tigecycline, GEH-High level gentamicin, STH-High level streptomycin, CIP-Ciprofloxacin, TCY-Tetracycline, MFX- Moxifloxacin, CLI-Clindamycin, CON-Positive Control.

No.1-No.9: the number of serial concentrations of antimicrobial agents.

7. Sterilized saline water
8. Mineral oil

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°C. 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 Broth should be used immediately once opened; store the Indicator Solution sealed at 2-8°C 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. After inoculum preparation, the inoculation should be completed within 20 minutes and sampled within 2 hours.

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

- 1) Add 1 mL sterilized saline water into turbidity tube, pick several pure culture isolates, mix it well in the turbidity tube, and prepare 0.5 McFarland bacterial suspension by using Nephelometer.
- 2) Add 100µL bacterial suspension into 1 vial Broth and mix well.
- 3) Add one drop of Indicator Solution into the Broth

For instrument

- 4) Place this Broth 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.

For manually

- 4) Transfer 100µL abovementioned Broth into each well and mix well.
- 5) Add one drop Mineral Oil into each well and cover the plate

with lid

- 6) Incubate the AST Plate at 35±2°C for 18-24 hours

3. Read results

For instrument

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

For manually

- Well CIP, Well MFX, the MIC results could be read for the well with no turbidity appearance comparing to the Well CON, and its corresponding lowest value of these antimicrobial agent in the reading card are their MIC results.
- Well ERY, Well NIT, Well SXT, Well LNZ, Well TGC, Well TCY, Well CLI, the MIC results could be read for the well with no or a few turbidity appearance comparing to the Well CON, and its corresponding lowest value of these antimicrobial agent in the reading card are their MIC results.
- MIC results of other wells could be read by comparing to the Well CON, the colors stay the same as blue, and its corresponding lowest value of these antimicrobial agent in the reading card are their MIC results.

Interpretation of Results

1. For the same antimicrobial agent, if there is a blue well among a serial of red wells or there is a red well among a serial of blue wells, then this well is a drift. The drift should be ignored when report the MIC. But if drift is ≥ 2 , the AST should be repeated.
2. It might be caused by bacterial contamination if the whole plate turns red, so the AST should be repeated.
3. It is invalid if there is no growth of bacterial or it remains blue of the Well CON, so the AST should be repeated.

Interpretive Category

The Interpretive Categorical Results (SIR) should be interpreted by referring to the latest MIC breakpoint interpretation standard published by CLSI⁴ or EUCAST⁵.

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 0.5-0.6 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.

Performance Characteristics

1. Phenotype screening of drug resistance

Staphylococcus aureus (ATCC29213) and *Enterococcus faecalis* (ATCC29212) are used as the control strain, MICs results should meet the criterion in Table 2.

Table 2: Criterion for Phenotype screening of drug resistance

Antimicrobial agents	<i>Staphylococcus aureus</i> (ATCC29213) (µg/mL)	<i>Enterococcus faecalis</i> (ATCC29212) (µg/mL)
High level gentamicin	/	No growth
High level streptomycin	/	No growth
Erythromycin/Clindamycin	No growth	/

Note: "/" means that this drug resistance experiment shouldn't be performed on the corresponding strain.

3 batches of Gram Positive bacteria AST, the results were met the requirements.

2. MIC testing

Staphylococcus aureus (ATCC29213) and *Enterococcus faecalis* (ATCC29212) are used as the control strain, MICs results of at least 19 out of 20 antimicrobial agents should meet the following criteria (shown in table 3 below).

Table 3: the MIC requirements range for other 20 antibacterial drugs.

Antimicrobial agents	<i>Staphylococcus aureus</i> (ATCC29213) (µg/mL)	<i>Enterococcus faecalis</i> (ATCC29212) (µg/mL)
Ceftaroline	≤0.5	0.25-2
Cefoxitin	≤4	>8
Penicillin	0.25-2	1-4
Oxacillin	≤0.5	8-32
Ampicillin	≤2	≤2
Daptomycin	≤1	1-4
Vancomycin	≤2	1-4
Teicoplanin	≤1	≤1
Linezolid	≤4	≤4
Erythromycin	≤1	1-4
Clindamycin	≤0.25	4-16
Ciprofloxacin	≤0.5	0.25-2
Moxifloxacin	≤0.12	≤0.5
Tetracycline	≤1	8-32
Tigecycline	≤0.25	≤0.12
Gentamicin	≤2	4-16
Furantoin	≤32	≤16

Trimethoprim-Sulfamethoxazole	≤0.5/9.5	≤0.5/9.5
Rifampicin	≤0.015	0.5-4
Oritavancin	≤0.12	≤0.03

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

3. Repeatability

Test the control strain *Enterococcus faecalis* (ATCC29212) with 10 plates from 1 batch of Gram Positive bacteria AST, the MICs results of at least 19 out of 20 antimicrobial agents should have a ≥90% consistency with the MIC requirements range. The results were met the requirements.

4. Between-batch precision

Test the control strain *Enterococcus faecalis* (ATCC29212) with 3 batches of Gram Positive bacteria AST (10 plates for each batch), the MICs results of at least 19 out of 20 antimicrobial agents should have a ≥85% consistency with the MIC requirements range. The results were met the requirements.

Literature References

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