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# Microbiological Assay

REF MD0101 / MD0102 / MD0103

*10 tests / 20 tests / 50 tests*

## Non-fermenting bacteria AST

*The Non-fermenting bacteria AST (antimicrobial susceptibility testing) is intended for in vitro determination of antimicrobial susceptibility of Non-fermenting bacteria.*



**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 | | do not reuse |
| | date of manufacture | | |

EC REP

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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 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

| Σ | 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 use

- AutoMic-i600

## Materials Required but not Provided

1. Micropipettor
2. Vibrator
3. Sterile tips or swabs
4. Nephelometer
5. Turbidity tubes
6. Incubator
7. Sterilized saline water
8. Mineral oil

Table 1: Distribution of antimicrobial agents

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ATM 1 | ATM 2 | ATM 3 | ATM 4 | ATM 5 | TZP 1 | TZP 2 | TZP 3 | TZP 4 | TZP 5 | TZP 6 | TZP 7 |
| CRO 1 | CRO 2 | CRO 3 | CRO 4 | CSL1 | CSL2 | CSL3 | CSL4 | CSL 5 | CZA 1 | CZA 2 | CZA 3 |
| CAZ 1 | CAZ 2 | CAZ 3 | FEP 1 | FEP 2 | FEP 3 | FEP 4 | FEP 5 | FEP 6 | FEP 7 | FEP 8 | FEP 9 |
| CAZ 4 | CAZ 5 | CAZ 6 | MEM 1 | MEM 2 | MEM 3 | MEM 4 | MEM 5 | MEM 6 | MEM 7 | MEM 8 | MEM 9 |
| SAM 1 | SAM 2 | SAM 3 | IPM 1 | IPM 2 | IPM 3 | IPM 4 | IPM 5 | IPM 6 | IPM 7 | IPM 8 | CON |
| AMK 1 | AMK 2 | AMK 3 | AMK 4 | AMK 5 | DOR 1 | DOR 2 | DOR 3 | DOR 4 | DOR 5 | DOR 6 | DOR 7 |
| GEN 1 | GEN 2 | GEN 3 | GEN 4 | POL 1 | POL 2 | POL 3 | POL 4 | POL 5 | POL 6 | POL 7 | POL 8 |
| TOB 1 | TOB 2 | TOB 3 | TOB 4 | TOB 5 | CHL 1 | CHL 2 | CHL 3 | CIP 1 | CIP 2 | CIP 3 | CIP 4 |
| TGC 1 | TGC 2 | TGC 3 | TGC 4 | TGC 5 | TGC 6 | TGC 7 | TGC 8 | LVX 1 | LVX 2 | LVX 3 | LVX 4 |
| TCY 1 | TCY 2 | TCY 3 | TCY 4 | MNO 1 | MNO 2 | MNO 3 | SXT 1 | SXT 2 | SXT 3 | SXT 4 | SXT 5 |

Abbreviation: ATM-Aztreonam, TZP-Piperacillin, CRO-Ceftriaxone, CSL-Cefoperazone/Sulbactam, CZA- Ceftazidime/avibatan, CAZ-Ceftazidime, FEP-Cefepime, MEM-Meropenem, IPM-Imipenem, SAM-Ampicillin/Sulbactam, AMK-Amikacin, DOR-Doripenem, GEN- Gentamicin, POL-Polymyxin B, TOB-Tobramycin, CHL-Chloramphenicol, CIP-Ciprofloxacin, TGC-Tigecycline, LVX-Levofloxacin, TCY-Tetracycline, MNO-Minocyline, SXT-Selectrin, CON-Positive Control.

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

## 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. Do not mix or use components from kits with different batch codes.
8. Micropipettor tips cannot be mixed to avoid contamination.
9. 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).
10. The AST Plate cannot be reused.

## 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 Broth should be used immediately once open; 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. After inoculum preparation, the inoculation should be completed within 20 minutes and sampled within 2 hours.

## Measurement Procedure

**1. Prepare the materials**

- Allow the inoculated samples to balance at room temperature for at least 30 minutes.
- Remove the kit from the refrigerated environment and return to room temperature before opening the package.

**2. Order tests**

1) Add 1mL 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℃ for 18-24 hours.

**3. Read results**

For instrument

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

For manually

- Well MEM, Well IPM, Well DOR: The MIC results could be read for the well with no turbidity appearance comparing to the Well CON (positive control), and its corresponding lowest value of the antimicrobial agent in the reading card are their MIC results.
- Well SXT: MIC results could be read by comparing to the Well CON, the first well that showed slight color change and significantly reduced turbidity compared with the Well CON (more than 80% reduction compared with the positive control). Its corresponding values of these antimicrobial agents 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 CLSI[4] or EUCAST[5].

## 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.

## Control Procedure

The recommended control requirement for this assay involves using Well CON (positive control）of the plate and reference strain to verify assay performance. The result is valid if the following assigned specification for the controls is met:
Well CON: there is growth of bacterial or the color changes of the Well CON.
Reference strain: conduct the operations described in **Measurement Procedure** on recommended control *Pseudomonas aeruginosa* (ATCC27853), MICs results of at least 21 out of 22 antimicrobial agents should fall into the acceptance range of MIC as in Table 2.

## Performance Characteristics

1. *Pseudomonas aeruginosa* (ATCC27853) is used as the control strain, MICs results of at least 21 out of 22 antimicrobial agents should meet the following criteria (shown in table 2 below).

Table 2: the MIC requirements range for 22 antibacterial drugs.

| Antibacterial agents | *Pseudomonas aeruginosa* (ATCC27853) (μg/mL) |
|---|---|
| Ceftriaxone | ≤64 |
| Cefrazidime | ≤4 |
| Cefepime | ≤4 |
| Cefoperazone/sulbactam | ≤16/8 |
| Meropenem | ≤1 |
| Imipenem | 1-4 |
| Aztreonam | ≤8 |
| Ampicillin/Sulbactam | >32/16 |
| Piperacillin/Tazobactam | ≤8/4 |
| Ciprofloxacin | ≤1 |
| Levofloxacin | ≤4 |
| Gentamicin | ≤2 |
| Amikacin | ≤4 |
| Tobramycin | ≤1 |
| Chloramphenicol | >32 |
| Polymyxin B | ≤2 |
| Selectrin | 8/152-32/608 |
| Tetracycline | 8-32 |
| Minocyline | ≥16 |
| Tigecycline | 4-16 |
| Doripenem | ≤0.5 |
| Ceftazidime/Avibatan | ≤4/4 |

Determine MIC for 3 batches of Non-fermenting bacteria AST, the results were met the requirements.

2. Repeatability: test the same control strain *Pseudomonas aeruginosa* (ATCC27853) with 10 plates from 1 batch of Non-fermenting bacteria AST, the MICs results of at least 21 out of 22 antimicrobial agents should have a ≥90% consistency with the MIC requirements range. The results were met the requirements.

3. Between-batch precision: test the control statin *Pseudomonas aeruginosa* (ATCC27853) with 3 batches of Non-fermenting bacteria AST(10 plates for each batch), the MICs results of at least 21 out of 22 antimicrobial agents should have a ≥85% consistency with the MIC requirements range. The results were met the requirements.

## Literature References

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

3. 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.

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

5. 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.