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Microbiological Assay

REF **ME0301 /ME0302 / ME0303**

10 tests/20 tests/50 tests

YEAST 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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Contact your local dealers for all product related questions in your local language

Intended use

The YEAST ID/AST is intended for the in vitro identification (ID) and in vitro determination of minimal inhibitory concentration (MIC) for yeast-like fungus.

Summary

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 fungus 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[1,2,3], 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 fungus). 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 YEAST 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 YEAST. The antimicrobial susceptibility test is based on broth microdilution method and redox method [4,5,6]. The ID/AST Plate is coated with varying concentrations of antimicrobial agents at appropriate well locations. The AST Broth with the targeted organism (density of 2.8-3.2 McFarland) and ID Broth are added with the colorimetric redox indicator (colorimetric oxidation-reduction) to make an inoculum suspension. The suspension and the ID/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 YEAST 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 Broth	6.0mL*10	6.0mL*20	6.0mL*50
Indicator Solution	3.0 mL*1	3.0 mL*1	3.0 mL*1

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

- ID/AST Plate

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

- AST Broth

The RPMI 1640 broth is used to enrich for targeted organism for detection.

- ID Broth

The ID Broth contains ammonium sulfate.

- 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	dGLU	GRT	dSOR)	SAC	dMLZ	PLE	lARA	dXYL	GAL	BXYL	ESC	URE

B	dGAL	dRAF	MAdG	GLYL	ERY	dCEL	ADO	2KG	BNAG1	BNAG2	PHOS	dMEL
C	dMAL	lRHA	NAG	XLT	LAC	TRE	ACT	CIT	lMLT	dGNT	NEG	CON
D	ANI1	ANI2	ANI3	ANI4	ANI5	ANI6	ANI7	ANI8	ANI9	AMB1	AMB2	AMB3
E	CAS1	CAS2	CAS3	CAS4	CAS5	CAS6	CAS7	CAS8	CAS9	AMB4	AMB5	AMB6
F	MIF1	MIF2	MIF3	MIF4	MIF5	MIF6	MIF7	MIF8	MIF9	AMB7	AMB8	AMB9
G	FLU1	FLU2	FLU3	FLU4	FLU5	FLU6	FLU7	FLU8	FLU9	FLU10	FCT1	FCT2
H	VOR1	VOR2	VOR3	VOR4	VOR5	VOR6	VOR7	VOR8	VOR9	VOR10	FCT3	FCT4
I	ITR1	ITR2	ITR3	ITR4	ITR5	ITR6	ITR7	ITR8	ITR9	FCT5	FCT6	FCT7
J	POS1	POS2	POS3	POS4	POS5	POS6	POS7	POS8	POS9	FCT8	FCT9	FCT10

Abbreviation: dGLU-glucose, GRT-glucuronate, dSOR-Sorbitol, SACsucrose, dMLZ -Songsanose, PLE-Palatinose, lARA-Arabic candy, dXYL-Xylose,BGAL-β-galactosidase,BXYL-β-xylosidase,ESC-quinone,URE-Urease,dGAL-Galactose,dRAF-Raffinose,MAdG-Methylα-D-glucopyranoside,GLYL-glycerin,ERY-Erythritol,dCEL-cellobiose,ADO-Calendula officinalis,2KG-Ketogluconate,BNAG1-glucosaminidase 1, BNAG2-glucosaminidase2, PHOS-Phosphatase, dMEL-honeybiose, dMAL-maltose, lRHA-L-rhamnose monohydrate, NAG-N-Acetyl-D-Glucosamine,XLT-Xylitol,LAC-lactose, TRE-D-Trehalose anhydrous, ACT-cycloheximide growth,CIT-Citrate, lMLT-Malate,dGNT-Gluconate, NEG-ID negative control, CON-AST positive control,ANI-Anidofungin, AMB-amphotericin B, CAS-Caspofungin,MIF-Micafungin,FLU-Fluconazole,FCT-5-Fluorocytosine,VOR-Voriconazole,ITR-Itraconazole,POS-posaconazol;

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 ID/AST Plate cannot be reused.
10. Do not mix or use components from kits with different batch codes.
11. Any serious incident shall be reported to manufacturer and competent authority of the Member State in which user and/or patient is established.

Storage

1. Store all components at 2-8℃. When stored as directed, all reagents are stable until the expiration date.
2. The ID/AST Plate should be used within 8 hours once opened; the AST Broth and ID Broth 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 fungus should be used within 24 hours at room temperature or 2-8℃. For the clinically cryopreserved pure fungus, it is recommended they should be used after activation and subculture.
3. The preparation and testing of fungus 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 3mL sterilized saline water into turbidity tube, pick several pure culture isolates, mix it well in the turbidity tube, and prepare 2.8-3.2 McFarland fungus suspension by using Nephelometer.
2) Add 3mL fungus suspension into 1 vial ID Broth.
3) Add one drop of Indicator Solution into the AST Broth.
4) Place this ID Broth with fungus, AST Broth with Indicator Solution and the ID/AST Plate in the designed position of instrument, 100μL inoculum is transferred into each well and the ID/AST Plate is incubated automatically.

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

For Identification test only

1) Add 3mL sterilized saline water into turbidity tube, pick several pure culture isolates, mix it well in the turbidity tube, and prepare 2.8-3.2 McFarland fungus suspension by using Nephelometer.
2) Add 3mL fungus suspension into 1 vial ID Broth and mix well.
3) Place the ID Broth with fungus and the ID/AST Plate in the designed position of the instrument, 100μL inoculum is transferred into the corresponding well and the ID/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 fungus suspension with sterilized saline.
2) Add 10μL fungus suspension and one drop of Indicator Solution into 1 vial AST Broth.
3) Place AST Broth with fungus and Indicator Solution and the ID/AST Plate in the instrument, 100μL inoculum is transferred into each well and the ID/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 fungus 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 strain is not pure; the fungus suspension does not reach the specified concentration; the strain activity is severely reduced; the strain database does not contain such bacteria.
4. When the antimicrobial susceptibility positive control well shows "-", it means that there is no fungus growth, the antimicrobial susceptibility result of the strain is invalid, and the test should be repeated.
5. When the MIC result is displayed as "-", it means that the result is abnormal, it is recommended to repeat the test.
6. 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 fungus stain test is required for the selection of the appropriate ID/AST Plate types. Accurate AST results may not be made without this test.
2. Use only well-isolated fungus colonies from one of the recommended primary isolation media. Use of mixed colonies could result in inaccurate AST interpretations.
3. A suspension equivalent of 2.8-3.2 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 ID/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.
Candida parapsilosis	ATCC22019
Candida krusei	ATCC6258
Cryptococcus neoformans	ATCC44104

2. MIC testing

Candida parapsilosis (ATCC22019) and *Candida krusei* (ATCC6258) were used as the control strains, MICs results of at least 8 out of 9 antimicrobial agents should meet the following criteria (shown in table 4 below).

Table 4: the MIC requirements range for other 9 antifungus drugs.

Antimicrobial agents	*Candida parapsilosis* (ATCC22019) (μg/mL)	*Candida krusei* (ATCC6258) (μg/mL)
Anidofungin	0.25-2	≤0.12
Caspofungin	0.25-1	0.12-1

Micafungin	0.5-2	0.12-0.5
amphotericin B	0.25-2	0.5-2
Itraconazole	0.06-0.5	0.12-1
Fluconazole	0.5-4	8-64
Voriconazole	≤0.12	0.06-0.5
posaconazole	0.03-0.25	0.06-0.5
5-Fluorocytosine	0.06-0.25	4-16

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

3. Repeatability

Test the control statin *Candida parapsilosis* (ATCC22019) and *Candida krusei* (ATCC6258) with 10 plates from 1 batch of YEAST ID/AST, the MICs results of at least 8 out of 9 antimicrobial agents should have a ≥90% consistency with the MIC requirements range. The results were met the requirements.

Literature References

1. 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.

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.