Amphotericin B HiMIC™ Plate Kit (AP) (0.06 - 4 mcg/ml)

MPK071

Antimicrobial Susceptibility Testing
For *In Vitro* Diagnostic use

Intended Use:
Amphotericin B HiMIC™ Plate Kit is ready to use Minimum Inhibitory Concentration (MIC) determination kit by broth microdilution method, capable of showing MIC's of Amphotericin B against test organism in the range of 0.06 mcg/ml to 4 mcg/ml following specified incubation condition.

Kit Contains:

No.	Content	1 KT	3KT
1.	96 Well HiMIC™ Plate	1 No.	3 No.
2.	HiMIC™ Diluent Tubes	12 No.	36 No.
3.	HiMIC™ Reading card	1 No.	3 No.
4.	HiMIC™ Incubation Tray	1 No.	3 No.

Introduction:
HiMIC™ Plate Kit is a broth microdilution method consisting of 96 well microtiter plate with detachable wells. It covers 7 two-fold dilutions in a single detachable well strip covering breakpoint scale which helps in reliable interpretation for Sensitive, Intermediate and Resistance detection with easy visual inspection, in compliance with CLSI and EUCAST guidelines. Detachable well strip is coated with gradient of antibiotics and growth medium which releases upon the rehydration. The detachable strip is coated with highest concentration in the top most well i.e. in "A" well and lowest concentration in the second last well i.e. "G" well whereas last well is a growth control i.e. "H" well. Each detachable well is given along with the antibiotic symbol at the top. HiMIC™ reading card is provided for easy interpretation of the MIC value by visible colour change.

METHOD AND USE OF HiMIC™ Plate Kit

- **Type of specimen**

Pure cultures should be derived from specimens obtained from patients prior to the initiation of antimicrobial therapy. Specimens can be of bacterial or fungal isolates derived from blood, urine, faeces, pus, CSF etc. Direct specimens should not be employed in this test. Refer procedure, which includes preparation of inoculum (1, 3).

- **Clinical specimen collection, handling and processing**

Follow appropriate techniques for handling specimens as per established guidelines. After use, contaminated materials must be sterilized by autoclaving before discarding (1,3).

- **Preparation of Inoculum**

For Yeast:

1. Inoculum is prepared by picking five distinct colonies of approximately 1mm from 24 hours old culture grown on Sabouraud Dextrose Agar (M063) and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline.
2. Vortex the resulting suspension and adjust the turbidity to yield 1 x 10^6 to 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

For Mould:

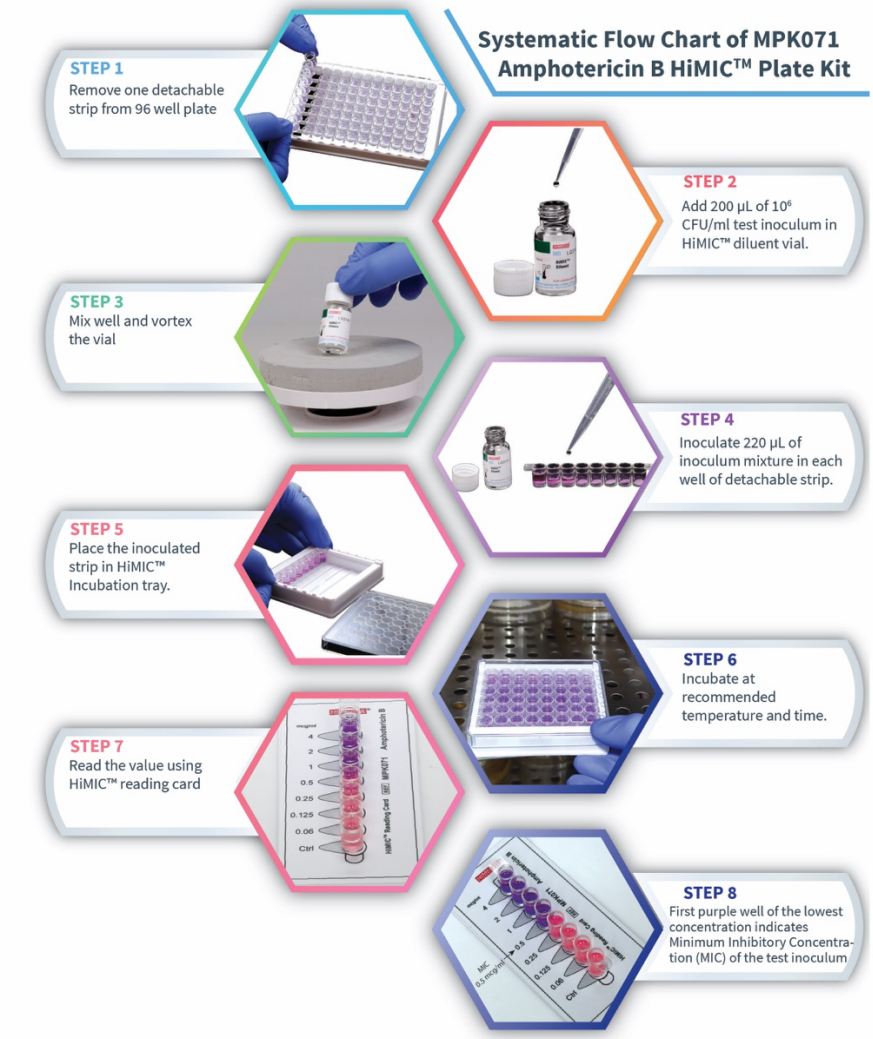
1. Inoculum is prepared by resuspending and scapping the conidia with approximately 5 ml sterile distilled water supplemented with 0.1%Tween 20.
2. Collect the scrapped suspension to the sterile tube and allow heavy particle to settle for 3-5 minutes.
3. Transfer upper homogenous suspension to a sterile tube and adjust turbidity to yield 1 x 10^6 to 5 x 10^6 cells /ml (i.e. 0.5 McFarland standard).

- **Test Procedure**

1. Remove the Amphotericin B HiMIC™ plate (HMP071) from Aluminum Zip Pouch.
2. Pick up the required number of detachable well strips and cover the remaining wells with the lid provided.
3. Inoculate 200 µL of 10^6 CFU/ml inoculum (i.e. 0.5 McFarland) into HiMIC™ diluent tube (LQ314II). (Note: Use one vial for single inoculum.)
4. Mix well and vortex the tube
5. Inoculate 220 µL of inoculum mixture from above tube into the all 8 wells i.e. "A" to "H" wells
6. Gently shake the well strip and keep it in an incubator tray provided with the kit.
7. Transfer the tray in the incubator under appropriate conditions.

MIC Reading:

1. Read the wells when visible color change from purple to pink will be observed (or Read the wells after appropriate incubation.)
2. Remove the detachable well Strip from incubator tray.
3. Place it on the HiMIC™ reading card in the proper direction
4. Read the MIC where lowest concentration of the purple colored well is observed



Warning and Precautions:

1. HiMIC™ Plate Kit is intended for *In vitro* diagnostic use only.
2. Although based on simple procedure, HiMIC™ Plate Kit should only be used by at least semi-trained personnel.
3. HiMIC™ Plate Kit, should be used strictly according to procedures described herein.
4. Performance of HiMIC™ Plate Kit depends on use of proper inoculum and control cultures and proper storage temperature.
5. Follow aseptic techniques and precautions against microbiological hazards should be used when handling bacterial or fungal specimen throughout the testing procedure.
6. Place the unused strips back to recommended temperature.

INTERPRETATION & QUALITY CONTROL (As per CLSI Guidelines) :

INTERPRETATION

Use following interpretive criteria for susceptibility categorization as per CLSI.

When testing	Incubation	Interpretative Criteria		
		≤ S	I	≥ R
Candida Spp	35-37°C for 24 - 48 hrs	1	-	2

Quality Control :

Quality control of Amphotericin B HiMIC™ Plate Kit is carried out by testing the strips with standard ATCC Cultures recommended by **CLSI**

Table 2: Following are the reference MIC values (mcg/ml) range for Amphotericin B.

Organism	Std. Quality Control limits (mcg/ml)	
	24 hours	48 Hours
C.parapsilosis ATCC 22019	0.25 – 0.5 – 1.0- 2.0	0.5 – 1.0 – 2.0 – 4.0
C. krusei ATCC 6258	0.5 – 1.0 – 2.0	1.0 – 2.0 – 4.0
C. albicans ATCC 90028	-	0.5 – 1.0 – 2.0
C. albicans ATCC 24433	-	0.25 – 0.5 – 1.0
C.parapsilosis ATCC 90018	-	0.5 – 1.0 – 2.0
C. tropicalis ATCC 750	-	0.5 – 1.0 – 2.0
Aspergillus flavus ATCC 204304	-	0.5 – 1.0 – 2.0 – 4.0
Aspergillus flavus ATCC MYA3631	-	1.0 – 2.0 – 3.0 – 8.0

INTERPRETATION & QUALITY CONTROL (As per EUCAST Guidelines) :

Interpretation:

Table 3: Use following interpretive criteria of Amphotericin B for susceptibility categorization as per **EUCAST.**

When testing	Incubation	Interpretive Criteria	
		≤ S	> R
Candida albicans, Candida parapsilosis, Candida glabrata, Candida krusei, Candida tropicalis	34 to 37°C for 18-24 hrs	1	1
Aspergillus. fumigatus, Aspergillus niger	34 to 37°C for 24-48 hrs	1	2

Quality Control:

Quality control of Amphotericin B HiMIC™ Plate Kit is carried out by testing the strips with standard ATCC Cultures recommended by **EUCAST** on suitable medium incubated appropriately.
Table 4 : Following are the reference MIC values (mcg/ml) range for Amphotericin B.

Organism	Incubation	Target MIC Value (mcg/ml)	Std. Quality Control limits (mcg/ml)
Candida krusei ATCC 6258	34 to 37°C for 18-24 hrs	0.25 – 0.5	0.125 – 0.25 – 0.5 – 1.0
Candida parapsilosis ATCC 22019	34 to 37°C for 18-24 hrs	0.25 – 0.5	0.125 – 0.25 – 0.5 – 1.0
Aspergillus fumigatus ATCC 204305	34 to 37°C for 24-48 hrs	0.5	0.25 – 0.5 – 1.0
Aspergillus flavus ATCC 204304	34 to 37°C for 24-48 hrs	1.0	0.5 – 1.0 – 2.0

Storage & Shelf Life:

1. Once the consignment is received, store the HiMIC™ Plate Kit at -20 to 8°C.
2. Use before expiry date on the label.
3. HiMIC™ Plate Kit left over from opened package must be kept dry.
4. Moisture should be prevented from penetrating into or forming within the package or storage container.
5. Check whether the batch number and expiry date are marked on the storage container.
6. Product performance is best within stated expiry period if correctly stored and handled.

Disposal:

After use, HiMIC™ Plate Kit and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2, 3).

Limitation of Test

HiMIC™ Plate Kit provides *In vitro* MIC values, which provides only a possible insinuation of pathogens potential in *In vivo* susceptibility. These values can be considered as a guide to therapy selection only after taking into consideration several other factors; and must be the sole decision and responsibility of the physician along with the clinical experience in treating the infection. These tests are comparable to the standards as per the given specifications and set of experiment standards as far as possible. Please refer to CLSI & EUCAST standards for detailed limitation of susceptibility test on the clinical use of an antibiotic in various therapeutic conditions.

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 1, Section 2.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition, Vol. 3, Section 15.
3. Jorgensen, J. H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Forth Edition. Vol.37 No.13, November-2017 CLSI document M27-Ed4.
5. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Third Edition. Vol.37 No.15, November- 2017 CLSI document M38-Ed3.
6. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. Vol.32 No.17, December 2012 CLSI document M27-S4.
7. Performance Standards of Antifungal Susceptibility Testing of Filamentous Fungi; Second Edition. M60-E02, Vol.40, No.8, Jan-2020.
8. Performance Standards of Antifungal Susceptibility Testing of Yeast; Second Edition. M61-E02, Vol.40, No.9, Jan-2020.
9. European Committee on Antimicrobial Susceptibility Testing, Routine and extended internal quality control for MIC determination and agar dilution for yeasts, molds and dermatophytes as recommended by EUCAST Version 5.0, valid from 2020-09-24.

10. European Committee on Antimicrobial Susceptibility Testing, Breakpoint tables for interpretation of MICs for antifungal agents Version 10.0, valid from 2020-02-04.

Packing:
Each Pack contains following material packed in a desired packing.

1) Amphotericin B HiMIC™ Plate Kit (HMP071) (1KT/3KT)
2) HiMIC™ Diluent tubes (LQ314II) (12NO./36NO)
3) HiMIC™ Reading Card. (1NO / 3NO)
4) HiMIC™ Incubation Tray (PW1378) (1NO / 3NO)
5) Package insert

Layout of plate
Product Code: MPK071
Product Name: Amphotericin B HiMIC™ Plate Kit
Concentration: 0.06 - 4 mcg/ml

	1	2	3	4	5	6	7	8	9	10	11	12
A	4	4	4	4	4	4	4	4	4	4	4	4
B	2	2	2	2	2	2	2	2	2	2	2	2
C	1	1	1	1	1	1	1	1	1	1	1	1
D	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
E	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
F	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
G	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
H	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control	Growth Control

CE

On receipt store at -20°C 8°C

IVD In vitro diagnostic medical device

Plot No. C-40, Road No. 21Y, MIDC, Wagale Industrial Estate, Thane (W) - 400604, Maharashtra, India

EC REP CEpartner4U, ESDOORNLAAN 13, 3951DB MAARN, NL www.cepartner 4u.eu

Do not use if package is damaged

Indicates a single sterile barrier system with protective packaging outside

Do not re-use