remel

RPMI 1640 AGAR w/ MOPS and 2% GLUCOSE

INTENDED USE

Remel RPMI 1640 Agar w/ MOPS and 2% Glucose is a plated medium recommended for use with antibiotic gradient-based systems for quantitative determination of susceptibility to antifungal agents when testing *Candida* spp. directly from colonies grown on nonselective media.

SUMMARY AND EXPLANATION

Fungal infections, especially invasive infections, are of great clinical relevance and pose therapeutic challenges due to increasing resistance to most commonly prescribed antifungals. Although fungi susceptibility testing is not widespread, yeast susceptibility testing is available to routine clinical laboratories.

Candida is the fourth most common cause of nosocomial bloodstream infections (BSI) in the United States.[1,2] Candidemia alone accounts for 8-10 infections per 100,000 patients every year and is associated with a mortality rate in excess of 30-50%.[3-7] The treatment of *Candida* BSI is often complicated by concerns of emerging resistance to antifungal agents.[8-12]

In the early 1990s, approximately two-thirds of candidal infections were caused by *Candida albicans*; by the end of the decade the number had decreased to about 50%. During the same period, BSI caused by other *Candida* spp., such as *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei* increased, accompanied by reduced susceptibility to common antifungal agents.[13] Any evidence to support the emergence of antifungal-resistant yeast isolates has been confounded by the lack of data from standardized susceptibility testing methods.[14]

Concerns of emerging resistance and the introduction of new antifungal agents have lead to increased demand for accurate species identification and antifungal susceptibility testing. Several authors and consensus groups have recommended routine antifungal susceptibility testing of *Candida* spp. isolated from sterile sites.[15,16] Recent studies examining the clinical utility of "real-time" antifungal susceptibility testing in the treatment of candidemia have demonstrated that when on-site testing is available, physicians frequently use the results to alter patient therapy.[17]

The Clinical and Laboratory Standards Institute (CLSI) has established reference methods for antifungal susceptibility testing, however, such methods may not be practical in the routine clinical laboratory. Among commercial alternatives is an antibiotic gradient-based system for quantitative determination of susceptibility to antifungal agents. RPMI-1640 was developed by Moore et al. at Roswell Park Memorial Institute. The formulation is based on the RPMI-1630 series of media utilizing a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins. RPMI-1640 medium has demonstrated wide applicability in cell culture and also as the reference method for antifungal broth microdilution recommended by CLSI. When properly supplemented with MOPS, glucose, and agar, RPMI-1640 has demonstrated accuracy for use with gradient-based systems with results comparable to that obtained with the CLSI reference method for testing *Candida* spp. against antifungal agents.[18-24]

PRINCIPLE

The use of RPMI-1640 medium supplemented with MOPS, agar, and glucose in the yeast susceptibility test in conjunction with antifungal gradient-based systems has been widely published and suggested as a reliable alternative to traditional broth dilution since the 1990s. The gradient method is based on a combination of the concepts of both dilution and diffusion tests, but differs from conventional disk methods by the use of a preformed, stable antibiotic gradient strip. When the strip is applied to the inoculated agar plate, there is an immediate release of the antibiotic into the agar matrix. A continuous and exponential gradient of antibiotic concentration is created beneath the carrier. After incubation a symmetrical inhibition ellipse centered along the carrier is seen. The zone edge intersects the strip at the MIC value given in µg/ml.

REAGENTS (CLASSICAL FORMULA)*

MOPS (morpholinepropanesulfonic acid)	35.0	g
Glucose	20.0	g
RPMI 1640	10.4	g
Agar	17.0	g
Demineralized Water	1000.0	ml

pH 7.0 ± 0.1 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) there is evidence of dehydration, (2) the product is contaminated, (3) the color has changed, (4) the expiration date has passed, or (5) there are other signs of deterioration.

SPECIMEN, COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.[25]

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) McFarland 0.5 standard or equivalent (REF R20410), (6) Broth or saline (0.85%), (7) McFarland 1 standard or equivalent (REF R20411) (mucoid yeast isolates), (8) Photometric device, (9) Antibiotic, gradient-based system, manufacturers insert, (10) Forceps, applicator.

PROCEDURE

1. Implement appropriate procedures to verify presumptive identification of the test isolate as *Candida* spp. RPMI 1640 Agar w/ MOPS and 2% Glucose is intended for use with pure, 24-48 hour cultures grown on nonselective media, not for inoculation directly from clinical specimens, organisms grown on selective media, or mixed cultures.
2. Allow plate and antibiotic gradient strip to equilibrate to room temperature prior to use. The agar surface should not have excess moisture prior to inoculation.
3. Prepare a suspension of the test isolate in broth or 0.85% saline from a pure, 24-48 hour culture on nonselective media.
4. Adjust the turbidity of the suspension visually to a 0.5 McFarland standard or equivalent, or use a photometric device. For mucoid organisms, use a 1 McFarland standard to compensate for turbidity associated with the strain's capsular material.
5. Inoculate agar plate within 20 minutes of preparing suspension.
6. Immerse a sterile cotton swab into the suspension. Rotate the swab against the side of the tube above the fluid level to remove excess fluid.
7. Inoculate the plate in three planes by rotating the plate approximately 60 degrees each time. Soak the swab again and repeat the streaking procedure a second time, swabbing the plate a total of 6 times.
8. Replace the lid and allow the excess moisture to be fully absorbed for approximately 15-20 minutes. The agar surface should be completely dry before applying the antibiotic gradient strip.
9. Using sterile forceps grip the handle of the antibiotic gradient strip or use an applicator. Position the strip with the MIC scale facing upwards and the concentration maximum nearest the periphery of the plate.
10. Make sure the strip is in complete contact with the agar surface (gently press the strip with sterile forceps to remove air pockets). Because some drug diffuses almost instantaneously, do not relocate a strip once it has come in contact with the agar surface.
11. Invert plate and place in incubator within 15 minutes of strip application.
12. Incubate plate in ambient air at 35°C for a full 24 hours.
13. After 24 hours incubation, examine the plate for a confluent lawn of growth and proceed with MIC determination. Confirm 24 hour readings at 36-48 hours.

INTERPRETATION

1. Read the MIC value at the point of intersection between the test isolate zone edge and the gradient strip.
2. The yeast species, antifungal agent, medium, incubation period, inoculum, and resistance mechanism can affect the appearance of the MIC endpoint. Azoles may give diffuse end-points. MICs should be read at the first point of significant inhibition or marked decrease in growth density. Use the principle of so-called 80% inhibition to visually select the MIC endpoint. For flucytosine, trailing endpoints should be read at approximately 90% inhibition of growth, ignoring faint hazes and minute colonies.
3. When high density growth occurs along the entire strip and no inhibition ellipse is seen, the MIC should be reported as greater than the highest value on the reading scale. When the inhibition ellipse is below the strip and the zone edge does not intersect the strip, the MIC should be reported as less than the lowest value on the reading scale.
4. Refer to the instructions for use (IFU) provided by the manufacturer of the antibiotic gradient-based system for complete interpretation of test results.

QUALITY CONTROL

All lot numbers of RPMI 1640 Agar w/ MOPS and 2% Glucose have been tested using the following quality control organisms and have been found to be acceptable. Controls should be included each time a susceptibility test is performed or weekly if satisfactory performance can be documented according to CLSI standards. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Candida albicans ATCC® 90028	Ambient, 48 h @ 35°C	Refer to interpretation guidelines in IFU provided by manufacturer of antibiotic gradient strip
Candida krusei ATCC® 6258	Ambient, 48 h @ 35°C	Refer to interpretation guidelines in IFU provided by manufacturer of antibiotic gradient strip
Candida parapsilosis ATCC® 22019	Ambient, 48 h @ 35°C	Refer to interpretation guidelines in IFU provided by manufacturer of antibiotic gradient strip

LIMITATIONS

1. Improper storage of antibiotic gradient strips may result in a loss of potency and false resistant interpretation.
2. Yeast suspensions must be prepared directly from colonies grown on nonselective media, and not by the inoculum growth method.
3. Incorrectly adjusted organism suspensions may adversely affect results.
4. Contamination or other changes in the control may yield erroneous results.
5. This product is intended for use in susceptibility determinations of *Candida* spp. with fluconazole, itraconazole, and flucytosine only. Performance data is not available for other drug/organism combinations.

PERFORMANCE CHARACTERISTICS[26]

Reproducibility studies were conducted in house and by 3 geographically distinct hospitals using 3 reference strains of *Candida* (*C. albicans* ATCC® 90028, *C. krusei* ATCC® 6258, and *C. parapsilosis* ATCC® 22019) and 24 clinical *Candida* isolates. Three independent lot numbers of media were tested in triplicate on three separate days with fluconazole, itraconazole, and flucytosine. There was 100% correlation with expected MIC values at 24 hours and 97.2% at 48 hours. An in-house reproducibility study with 8 clinical isolates with pre-determined MICs yielded 100% category agreement and 95.4% essential agreement. Additional testing of 16 clinical isolates by two study sites with known phenotypes yielded 90.5% category agreement and 92.9% essential agreement. Of 24 clinical strains evaluated (in-house and by two trial centers), 11 isolates had some degree of resistance or co-resistance to either of the antifungals (18 different phenotype combinations). All nonsusceptible phenotypes (18/18) were detected, and 17 of 18 (94.4%) classified into correct susceptibility categories.

BIBLIOGRAPHY

1. Edmond, M.B., S.E. Wallace, D.K. McClish, M.A. Pfaller, R.N. Jones, and R.P. Wenzel. 1999. Clin. Infect. Dis. 29:239-244.
2. Centers for Disease Control and Prevention. 2000. Monitoring Hospital-Acquired Infections to Promote Patient Safety--United States, 1990-1999. Morbid. Mortal. Weekly Rep. 49:149.
3. Hajjeh, R.A., A.N. Sofair, L.H. Harrison, G.M. Lyon, B.A. Arthington-Skaggs, S.A. Mirza, M. Phelan, J. Morgan, W. Lee-Yang, M.A. Ciblak, L.E. Benjamin, L.T. Sanza, S. Huie, S.F. Yeo, M.E. Brandt, and D.W. Warnock. 2004. J. Clin. Microbiol. 42:1519-1527.
4. Kao, A.S., M.E. Brandt, W.R. Pruitt, L.A. Conn, B.A. Perkins, D.S. Stephens, W.S. Baughman, A.L. Reingold, G.A. Rothrock, M.A. Pfaller, R.W. Pinner, and R.A Hajjeh. 1999. Clin. Infect. Dis. 29:1164-1170.
5. Rees, J.R., R.W. Pinner, R.A. Hajjeh, M.E. Brandt, and A.L. Reingold. 1998. Clin. Infect. Dis. 27:1138-1147.
6. Gudlaugsson, O., S. Gillespie, K. Lee, J. Vande Berg, J. Hu, S. Messer, L. Herwaldt, M. Pfaller, and D. Diekema. 2003. Clin. Infect. Dis. 37:1172-1177.
7. Wey, S.B., M. Mori, M.A. Pfaller, R.F. Woolson, and R.P. Wenzel. 1988. Arch. Intern. Med. 148:2642-2645.
8. Miller, L.G., R.A. Hajjeh, and J.E. Edwards, Jr. 2001. Clin. Infect. Dis. 32:1110.
9. Pelz, R.K., P.A. Lipsett, S.M. Swoboda, M. Diener-West, J.M. Hammond, and C.W. Hendrix. 2000. Surg. Infect. 1:273-281.
10. Baddley, J.W., M. Patel, M. Jones, G. Cloud, A.C. Smith, and S.A. Moser. 2004. Diagn. Microbiol. Infect. Dis. 50:119-124.
11. Lortholary, O., A. Charlemagne, F. Bastides, P. Chevalier, A. Datry, M.F. Gonzalves, G. Michel, P. Tilleul, B. Veber, and R. Herbrecht. 2004. J. Antimicrob. Chemother. 54:456-464.
12. Pfaller, M.A., D.J. Diekema, and D.J. Sheehan. 2006. Clin. Microbiol. Rev. 19:435-447.
13. Pfaller, M.A., D.J. Diekema, R.N. Jones, H.S. Sader, A.C. Fluit, R.J. Hollis, and S.A. Messer. 2001. J. Clin. Microbiol. 39:3254-3259
14. Trick, W.E., S.K. Fridkin, J.R. Edwards, R.A. Hajjeh, R.P. Gaynes. 2002. Clin. Infect. Dis. 35:627-630.
15. Denning, D.W. 2003. Lancet. 362:1142-1151.
16. Hospenthal, D.R., C.K. Murray, and M.G. Rinaldi. 2004. Diagn. Microbiol. Infect. Dis. 48:153-160.
17. Hadley, S., J.A. Martinez, L. McDermott, B. Rapino, and D.R. Snydman. 2002. J. Antimicrob. Chemother. 49: 415-419.
18. Espinel-Ingroff, A. 1994. Diagn. Microbiol. Infect. Dis. 19:217-220.
19. Espinel-Ingroff, A., M. Pfaller, M.E. Erwin and R.N. Jones. 1996. J. Clin. Microbiol. 34:848-852.
20. Maxwell, M.J., S.A. Messer, R.J. Hollis, D.J. Diekema, and M.A. Pfaller. 2003. J. Clin. Microbiol. 41:97-99.
21. Morace, G., G. Amato. F. Bistoni. G. Fadda. P. Marone, M.T. Montagna, S. Oliveri, L. Polonelli, R. Rigoli, I. Mancuso, S. La Face, L. Masucci, L. Romano, C. Napoli, D. Tato, M.G. Buscema, C.M. Belli, M.M. Piccirillo, S. Conti, S. Covan, F. Fanti, C. Cavanna, F. D'Alo, and L. Pitzurra. 2002. J. Clin. Microbiol. 40:2953-2958.
22. Pfaller, M.A., S.A. Messer, A. Bolmstrom, F.C. Odds, and J.H. Rex. 1996. 34:1691-1693.
23. Pfaller, M.A., S.A. Messer, A. Houston, K. Mills, A. Bolmstrom, and R.N. Jones. 2000. J. Clin. Microbiol. 38:3715-3717.
24. Wanger, A., K. Mills, P.W. Nelson, and J.H. Rex. 1995. Antimicrob. Agents Chemother. 39:2520-2522.
25. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington DC.
26. Data on file. 2007. Remel Inc., Lenexa, KS.

PACKAGING

RPMI 1640 Agar w/ MOPS and 2% Glucose:
REF R04067, 15 X 150 mm Plate 10/Pk

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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