510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K072827

B. Purpose for Submission:

To obtain substantial equivalence determination for the RPMI 1640 Agar with MOPS and 2% Glucose Antifungal Susceptibility Test Medium

C. Measurand:

Susceptibility of fungal isolates

D. Type of Test:

Antifungal Susceptibility Test Medium for use with antibiotic gradient-based systems

E. Applicant:

Remel, Inc.

F. Proprietary and Established Names:

Susceptibility Test Medium, RPMI 1640 Agar with MOPS and 2% Glucose

G. Regulatory Information:

1. Regulation section:

   21 CFR Part 866.1700 Culture medium for antimicrobial susceptibility tests

2. Classification:

   Class II

3. Product code:

   MJE - Culture media, antifungal susceptibility test

4. Panel:

83 Microbiology

1. Intended use(s):

RPMI 1640 Agar with 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* species directly from colonies grown on nonselective media.

2. Indication(s) for use:

RPMI 1640 Agar with MOPS and 2% Glucose antifungal susceptibility test media is indicated for use with antibiotic gradient systems for quantitative determination of susceptibility to fluconazole, itraconazole, and flucytosine when testing *Candida* species.

3. Special conditions for use statement(s):

Prescription use only.

For antifungal testing, due to trailing effect, minimum inhibitory concentrations (MIC's) should be read at approximately 90% inhibition of growth, ignoring faint hazes and minute colonies for flucytosine and 80% inhibition for fluconazole and itraconazole.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals.

4. Special instrument requirements:

N/A

I. Device Description:

RPMI 1640 is a modified plated media developed from the RPMI 1630 media series, which is supplemented with 35 grams of morpholinepropanesulfonic acid (MOPS) per liter of broth media, 2% glucose and agar. Antifungal agents are impregnated on a preformed rectangular plastic strip (5 x 60 mm). One side of the strip carries a letter code designating the identity of the antifungal agent and is calibrated in terms of MIC values in micrograms/ml (µg/ml). A predefined and exponential gradient of the dried and stabilized antifungal agent, covering a continuous concentration range across 15 two fold dilutions of a conventional MIC method, is immobilized on the opposite surface of the carrier strip. The concentration maximum is at the opposite end from the concentration minimum. When applied to an inoculated RPMI-1640 agar plate, the agent is immediately released into the agar matrix beneath the carrier. After aerobic incubation at 35° C from 24 to 48 hours a symmetrical inhibition ellipse

centered along the carrier is seen. The zone edge intersects the strip at the minimum inhibitory concentration (MIC) value given in micrograms/ml (µg/ml).

J. Substantial Equivalence Information:

1. Predicate device name
   Mueller Hinton Agar with 2% NaCl

2. Predicate 510(k) number
   K960313

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Atmosphere	Aerobic	Same
Test medium	Gradient strip(s) placed on solid agar	Same

Differences		
Item	Device	Predicate
Incubation time and temperature	35° C for 24 hours with MIC confirmation at 36 - 48 hours	30 - 35° C for 24 hours
Inoculum concentration	*Candida* species, suspension adjusted to 0.5 McFarland standard, or 1 McFarland standard for mucoid strains	*Staphylococcus* species suspension adjusted to 0.5 McFarland standard
Inoculum technique	Plate swabbed 6 times for confluent growth	Plate swabbed 3 times for confluent growth
Interpretation	MIC's should be read at approximately 90% inhibition of growth, ignoring faint hazes and minute colonies for Flucytosine and 80% inhibition for Fluconazole and Itraconazole.	MIC is read at the end point where there is complete inhibition by oxacillin

K. Standard/Guidance Document Referenced (if applicable):

M27-A2, "*Reference Method for Broth Dilution Antifungal Susceptibility Testing of*

Yeasts; Approved Standard – Second Edition", NCCLS, 2002.

L. Test Principle:

RPMI 1640 was developed by Moore et al. at Roswell Park Memorial Institute (RPMI). The formulation is based on the RPMI-1630 media series which utilizes a bicarbonate buffering system and differing amounts of amino acids and vitamins. RPMI-1640 (liquid) medium is widely used in cell culture and more recently as the reference method for antifungal broth microdilution recommended by the Clinical Laboratory Standards Institute (CLSI). This formulation of RPMI 1640 is supplemented with 35 grams of morpholinepropanesulfonic acid (MOPS) per liter of broth media, 2% glucose and agar. The antibiotic gradient-based method is based on a combination of the concepts of both dilution and diffusion principles for susceptibility testing. Antibiotic/antifungal agents are impregnated on a preformed plastic strip, which, when applied to an inoculated agar plate, are immediately released into the agar matrix. A continuous and exponential gradient of antibiotic/ antifungal concentration is created beneath the carrier. After incubation at 35° C a symmetrical inhibition ellipse centered along the carrier is seen. The zone edge intersects the strip at the minimum inhibitory concentration (MIC) value given in micrograms/ml (µg/ml).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility of QC organisms:*
   Three different lots of Etest®/RPMI-1640 media agar with MOPS and 2% Glucose were tested in triplicate for three consecutive days at one internal site and at 3 external clinical trial sites for intra-site and inter-site reproducibility. There were 648 results generated at 24 hours, and a total of 729 results generated at 48 hours, respectively, using three fungal QC ATCC organisms, at the external clinical sites. A total of 72 results at 24 hours and 81 results at 48 hours were obtained at the internal site. Fluconazole and Itraconazole MIC results demonstrated >95 % reproducibility. Flucytosine MIC's demonstrated >95 % reproducibility after 24 hours of incubation. There were 10 *C. albicans* ATCC 90028 MIC values that read one dilution above the expected range after 48 hours of incubation, only at one external study site. There were 12 MIC's at the same site that could be read as either 2 or 4 µg/ml. For consistency all 12 readings were analyzed at 4 µg/ml, which were within the expected MIC range. The Reproducibility Study is summarized in the tables

below.

24 hours	Fluconazole	Itraconazole	Flucytosine	Total
External sites	162/162*	243/243	243/243	648/648
Internal site	18/18	27/27	27/27	72/72
Total	180/180	270/270	270/270	720/720
Percentage in Range	100%**	100%	100%	100%
48 hours				
External sites	243/243	242/243	221/243	706/729
Internal site	27/27	27/27	27/27	81/81
Total	270/270	269/270	248/270	787/810
Percentage in Range	100%	99.6%	91.9%	97.2%

* Number of readings in expected MIC range / total number of readings
**Percentage of readings within the expected MIC range

The results for which the Flucytosine MIC values were above the antifungal gradient recommended QC ranges were obtained all those same runs. The subjective reading of antifungal susceptibility test results due to the trailing effect of Flucytocine at 48 hours of incubation was due to operator-related interpretation.
Although the number of reproducible Flucytosine results at 48 hours was below the expected level at one site, the combined reproducibility for all three antifungal agents at the three external sites and the one internal site was greater than 95%. Therefore, the combined reproducibility data are acceptable.

b. *Linearity/assay reportable range:*

N/A

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The CLSI recommended QC organisms *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were tested at each clinical trial site using RPMI-1640 media agar with MOPS and 2% Glucose, and the antifungal gradient-based method. The CLSI and Etest® QC expected ranges for yeast microdilution testing for each QC organism are provided below.

Expected QC Ranges for Yeast Microdilution Testing in µg/ml

Agent	*C.parapsilosis* ATCC 22019 CLSI	*C. parapsilosis* ATCC 22019 Etest®	*C. krusei* ATCC 6258 CLSI	*C. krusei* ATCC 6258 Etest®**	*C. albicans* ATCC 90028 Etest®
Fluconazole					
24 hours	0.5 - 4	1 - 4	8 - 64	N/A	0.125-0.5
48 hours	1 – 4	1 - 4 (8)*	16 - 128	128 - >256	0.125-0.5
Itraconazole					

24 hours	0.12 - 0.5	0.032 - 0.25	0.12 – 1	0.125 – 0.5	0.032 – 0.125
48 hours	0.12 - 0.5	0.064 - 0.25	0.25 - 1	0.25 - 1	0.064 – 0.25
Flucytosine* **					
24 hours	0.06 - 0.25	0.064 - 0.25	4 - 16	≥ 32	0.125 – 1
48 hours	0.12 - 0.5	0.064 - 0.25	8 - 32	≥ 32	0.5 - 2

* Occasionally, isolated colonies may grow up to 8 µg/ml.

** *C. krusei* are assumed to be intrinsically resistant to azoles. CLSI Expected Range QC differs from Etest. The *C. krusei* was removed from the current Etest Package Insert (Table 2) because the QC ranges/values are off-range. This isolate is no longer recommended as a useful QC strain for Fluconazole or Flucytosine by AB Biodisk.

***Flucytosine: Interpretive criteria and recommended QC organisms as described in CLSI document M27-A2 are the same as those referenced in the FDA approved drug label updated on 08/10/2006.
N/A = Not applicable

d. Detection limit:

N/A

e. Analytical specificity:

N/A

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison:

A total of 16 fungal clinical isolates with known MIC results were tested once at two clinical sites with the RPMI 1640 agar with MOPS and 2% Glucose, with gradient-based methodology. The original test method used at Site 1 was broth microdilution, prepared according to the CLSI reference method. The original test method at Site 2 was the antibiotic gradient method, tested on in-house RPMI agar plates prepared at the site. This data is presented for comparative purposes only and is not included in the reproducibility study calculations. The summary of the results are presented in the table below.

Additional Clinical Isolates	**N**	**% EA**	**% CA***
Fluconazole			
Site 1	6	100	100
Site 2	10	100	90

Total	**16**	**100**	**93.8**
Itraconazole			
Site 2	10	90	80
Total	**10**	**90**	**80**
Flucytosine			
Site 1	6	83.3	100
Site 2	10	90	90
Total	**16**	**87.5**	**93.8**
Combined Total		**92.9**	**90.5**

* Interpretation criteria per CLSI document M27-A2

Essential Agreement (EA) was calculated when the results for the antifungal drug MIC were within +/- two doubling dilutions for each agent.

The overall correlation between RPMI 1640 agar with MOPS and 2% Glucose using gradient-based methodology MIC's with previously known MIC results obtained using two different test methods was good.

There were 7 results that did not correlate, with six of the seven results observed at one site (Site 2). A summary of the results that did not correlate is provided in the table below.

	Site	**Previously known MIC**	**CLSI Interpretation**	**Etest® MIC**	**CLSI Interpretation**
Isolate #5	1	Flucytosine - 0.023	S	0.25	S
Isolate #1	2	Fluconazole - 32	SDD	128	R
Isolate #7	2	Itraconazole – 1	R	32	R
Isolate #8	2	Itraconazole – 0.06	S	0.25	SDD
Isolate #10	2	Itraconazole – 0.12	S	0.5	SDD
Isolate #6	2	Flucytosine – 16	I	32	R
Isolate #9	2	Flucytosine – 0.06	S	0.01	S

S = Susceptible; SDD = Susceptible-Dose Dependent; I = Intermediate; R = Resistant

Three of the seven results remained within CA. However, there was a trend for all 7 gradient-based methodology results to show slightly higher MIC's than the pre-determined MIC. This observation may be due to the well documented trailing

effect and operator related interpretation associated with antifungal susceptibility testing using this testing method.

The combined performance for the three antifungal agents tested at two clinical sites demonstrated an EA of 92.9% and a CA of 90.5%, which are acceptable.

Detection of non-susceptible clinical isolates

The primary role of antifungal susceptibility testing is to identify resistant isolates among susceptible populations. Of the 24 total *Candida* species evaluated during the reproducibility study, there were 11 isolates that demonstrated resistance to one or more antifungal agent, both internally and at one clinical site (Site 2).

There were a total of 18 different resistance phenotype combinations detected. The gradient-method detected 100% (18/18) of the non-susceptible isolates. There were 17 of 18 (94.4%) *Candida* species isolates grouped into the appropriate interpretive category, as either SDD or R. All non-susceptible isolates were identified, the data are acceptable and the performance was good.

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

N/A

b. Clinical specificity:

N/A

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

N/A

5. Expected values/Reference range for *Candida* species:

CLSI Interpretive Criteria Category	Fluconazole	Itraconazole	Flucytosine**
Susceptible	≤ 8	≤ 0.125	≤ 4
Susceptible – Dose Dependent*	16 – 32	0.25 – 0.5	-
Intermediate	-	-	8 – 16
Resistant	≥ 64	≥ 1	≥ 32

*Susceptible – Dose Dependent requires achieving maximum possible blood level for azoles.
**Flucytosine: Interpretive criteria and recommended QC organisms as described in CLSI document M27-A2 are the same as those referenced in the FDA approved drug label updated on 08/10/2006.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The information submitted in this premarket notification is complete and supports a substantial equivalence determination.