

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number:

k040560

B. Purpose for Submission:

AST testing of Candida spp.

C. Analyte:

Fluconazole at 0.016-256 ug/mL

Itraconazole at 0.002-32 ug/mL

Flucytosine at 0.002-32 ug/mL

D. Type of Test:

Quantitative AST growth based detection

E. Applicant:

AB BIODISK

F. Proprietary and Established Names:

Etest® for Antifungal Susceptibility Testing

G. Regulatory Information:

1. Regulation section:
866.1640 Antimicrobial Susceptibility Test
2. Classification:
II
3. Product Code:
NGZ Susceptibility testing -antifungal
4. Panel:
83 Microbiology

H. Intended Use:

1. Intended use(s):
Etest® is an agar-based gradient technique for quantitative antifungal susceptibility testing of Candida species. It uses a predefined concentration gradient of the specific antifungal agent to determine the Minimum Inhibitory Concentration (MIC) in ug/mL inhibiting the growth of the test organism under defined conditions.
2. Indication(s) for use:
This indication is for the addition of Etest® for MIC determination of Fluconazole (0.016-256 ug/mL), Itraconazole MIC (0.002-32 ug/mL) and Flucytosine MIC (0.002-32 ug/mL) with Candida species.
3. Special condition for use statement(s):
For prescription use

Preliminary results available at 24 hours with final results available at 48 hours.

4. Special instrument Requirements:
Manual readings only.

I. Device Description:

Etest® consists of a thin, inert and non-porous plastic strip, 5mm wide and 60 mm long. One side of the strip carries a two-letter code designating the identity of the antifungal agent and is calibrated with MIC values in terms of ug/mL, a predefined and exponential gradient of the dried and established antifungal agent which covers a continuous concentration range across two fold dilutions.

J. Substantial Equivalence Information:

1. Predicate device name(s):
YEASTONE®
2. Predicate K number(s):
K991810
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	Quantitative susceptibility to antifungal agents for Candida species	same
Incubation	35°	35°
Inoculation	Isolated colonies of Candida species	same
Differences		
Item	Device	Predicate
Technology	Predefined and preformed antifungal gradient on a plastic strip-growth based	Broth mico-dilution with colorimetric growth indicator- growth based
Format	Single gradient strip per antifungal agent	Micro tray with all three antifungal agents
Medium	RPMI 1640 as described in NCCLS M27A with 2% glucose and 1.5% Bacto agar	Sensititre® yeast susceptibility inoculum broth

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

“Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA”, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard (M27-A2)

L. Test Principle:

The Etest® gradient technology is based on a combination of the concepts of dilution and diffusion test methods for susceptibility testing. Etest® directly quantifies antifungal susceptibility in terms of discrete MIC values. When the Etest® strip containing the antibiotic is applied to an inoculated agar plate, the antifungal agent is immediately released from the plastic surface into the agar. A predefined, continuous gradient of antifungal concentrations is created and maintained directly underneath the strip. After incubation whereby growth becomes visible, a symmetrical inhibition

ellipse centered along the strip will be seen. The inhibition zone edge intersects the strip of the MIC value in ug/mL of the agent.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility was determined by testing 25-26 Candidid spp. one time at each of three sites. The mode was determined and then the reproducibility was calculated based on plus or minus one well of the mode.

Antifungal agent	Reproducibility at 24 hour Etest readings	Reproducibility at 48 hour Etest readings
Fluconazole	73/78 = 93.6%	78/78 = 100%
Itraconazole	74/75 = 98.7%	73/75 = 97.3%
Flucytosine	75/75 = 100%	75/75 = 100%

b. Linearity/assay reportable range:

Not applicable

c. Traceability (controls, calibrators, or method):

Quality control was performed each day of testing on both the reference method and the test method. For those results that are different from the NCCLS broth microdilution recommended ranges, additional test data was provided to support the new ranges.

Fluconazole

ORGANISM	Concentration	Reference	Etest®		
			24h	48h*	
C. parapsilosis ATCC 22019 Recommended Range at 48h NCCLS 1-4 ug/mL Etest 1-4 ug/mL	0.5				*
	1		15		7
	2	51	32	35	501
	4	9	14	26	413
	8			2	109
C. krusei ATCC 6258 NCCLS range 16-128 (48h) Etest range none	8				
	16				
	32	32	19		
	64	36	32		
	128		5	1	
	256		3	66	
					*
C. albicans ATCC 90028 Recommended Range at 48h NCCLS none Etest 0.125-0.5 ug/mL	0.064				
	0.125	1		12	109
	0.25	38	37	47	668
	0.5	10	15	3	154
	1		7		

* For ranges that are different than the NCCLS broth microdilution recommendations additional studies were performed at multiple sites using different lots of Etest® and medium batches.

C. krusei is considered intrinsically resistant to fluconazole and does not make a good control isolate for this drug.

Itraconazole

ORGANISM	Concentration	Reference	Etest®		
			48h	24h	48h*
<i>C. parapsilosis</i> ATCC 22019 Recommended Range at 48h NCCLS .12-.5 ug/mL Etest .064-.25 ug/mL	0.032		7		*
	0.064	5	22	14	222
	0.125	29	21	26	533
	0.25	16		10	298
	0.5				
<i>C. krusei</i> ATCC 6258 Recommended Range at 48h NCCLS 0.25-1 Etest range 0.25-1	0.125	2	26	4	
	0.25	32	14	21	
	0.5	15	7	21	
	1	1	3	4	
	2				
<i>C. albicans</i> ATCC 90028 Recommended Range at 48h NCCLS none Etest 0.064-0.25ug/mL	0.032	23	23	8	
	0.064	20	25	27	307
	0.125	5		15	443
	0.25				84

* For ranges that are different than the NCCLS broth microdilution recommendations additional studies were performed at multiple sites using different lots of Etest® and medium batches.

Itraconazole ranges for 24 hour readings are slightly more susceptible and the clinical performance suggests that a 48 hour reading correlates better with the NCCLS reference method.

Flucytosine

ORGANISM	Concentration	Reference	Etest®		
			48h	24h	48h*
C. parapsilosis ATCC 22019 Recommended range at 48h NCCLS 0.12-0.5ug/mL Etest 0.064-0.25ug/mL	0.064		18	12	45*
	0.125	23	29	31	321
	0.25	27	5	9	167
	0.5				
	1				
C. krusei ATCC 6258 NCCLS range 8-32 (48h) Etest range none	4				
	8	3			
	16	24			
	32	25	52	52	
C. albicans ATCC 90028 Recommended Range at 48h NCCLS none Etest 0.5-2 ug/mL	0.125		1		*
	0.25	3	15	7	5
	0.5	13	19	21	66
	1	36	17	24	399
	2				67

* For ranges that are different than the NCCLS broth microdilution recommendations additional studies were performed at multiple sites using different lots of Etest® and medium batches.

A 0.5 McFarland is used to determine the correct inoculum. Colony counts were performed periodically at each site to demonstrate that the inoculum procedure results in the expected CFU/ml.

The no growth rate was <5% for all antifungal agents tested.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Not applicable

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Clinical laboratory studies were performed at three or more clinical laboratories and compared to the NCCLS broth microdilution method as described in M27 A2. Seventy five challenge strains were tested at one site and were included in the evaluation. Both tests were incubated in ambient air at 35°. The reference method was read at 48 hours and the Etest® was read at 24 hours for a preliminary result with a final reading at 48 hours. A two concentration allowance was used for this comparison due to the difficulty in reading the Essential Agreement (EA) for both the reference method

and the Etest®. Category Agreement (CA) was determined based on the interpretation of each test.

	total	% EA +/- 2 well	CA	%CA	#R	min	maj	vmj
Fluconazole								
Candida spp. 24 h*	591	96.6	546	92.6	25	45	1	0
Candida spp. 48h	596	97.1	522	87.6	25	69	5	0
<ul style="list-style-type: none"> Etest at 48h is slightly more resistant than the NCCLS reference method <i>C. glabrata</i> is responsible for the majority of minor and major errors 								
Itraconazole								
Candida spp. 24h*	713	88.8%	585	82%	111	119	1	8
Candida spp. 48h	722	94.7	608	84.2	112	114	0	1
<ul style="list-style-type: none"> The lower than expected category agreement (CA) for itraconazole at both 24 and 48 hour readings are due mainly to minor errors in all species. Due to the large number of very major errors itraconazole should not be read at 24 hour 								
Flucytosine								
Candida spp. 24h*	566	93.6%	546	96.5	30	20	0	0
Candida spp. 48h	572	98.3	550	96.2	30	22	0	0
<ul style="list-style-type: none"> Etest is slightly more susceptible than the NCCLS reference method 								

EA-Essential Agreement

maj-major discrepancies

CA-Category Agreement

vmj-very major discrepancies

R-resistant isolates

min- minor discrepancies

* Readings include only those that were readable at 24h. Majority of the times the 24 hour readings are more susceptible than the 48 hour. In most cases it results in a minor error except for itraconazole which would result in a high very major rate at 24 hour. Confirming these readings at 48 hours removed the errors.

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Fluconazole	$\leq 8, 16-32, \geq 64$ ug/mL
Itraconazole	$\leq 0.125, 0.25-0.5, \geq 1$ ug/mL
Flucytosine	$\leq 4, 8-16, \geq 32$ ug/mL

N. Conclusion:

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