510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221198

B Applicant

Thermo Fisher Scientific

C Proprietary and Established Names

Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128ug/mL

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
NGZ	Class II	21 CFR 866.1640 - Antimicrobial Susceptibility Test Powder	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain clearance for the YeastOne Susceptibility System with Fluconazole for a dilution range of 0.12 – 128 µg/mL and with revised breakpoints for *C. albicans*, *C. glabrata*, and *C. parapsilosis.*

B Measurand:

Fluconazole at concentrations of 0.12 – 128 µg/mL

C Type of Test:

Quantitative Antifungal Susceptibility test, growth based

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Sensititre YeastOne Susceptibility System is an in vitro diagnostic product for clinical susceptibility testing of Candida spp.

This 510(k) is for Fluconazole with new FDA breakpoints and indications for testing Candida spp on the Sensititre YeastOne Susceptibility System.

Fluconazole has been shown to be active both clinically and in vitro against the following organisms according to the FDA drug label:

Candida albicans
Candida glabrata
Candida parapsilosis

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

Studies of fluconazole with Candida spp. were performed using the AIM autoinoculator inoculation method and the VIZION reading method only. The use of alternative inoculation methods or alternative reading methods when testing fluconazole have not been evaluated.
Due to the low performance of fluconazole with C. tropicalis, isolates of C. tropicalis should be tested with an alternate method.
The performance of the Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128 µg/mL was not evaluated with challenge isolates harboring resistance mechanisms listed in the FDA drug label.

D Special Instrument Requirements:

Sensititre VIZION

IV Device/System Characteristics:

A Device Description:

The Sensititre YeastOne Susceptibility System is a micro-version of the broth dilution susceptibility test performed in multi-well microtiter plates. Various antifungal agents are serially diluted to concentrations bridging the range of clinical interest in autoclaved diluent which contains a colorimetric growth indicating compound.

A standardized organism suspension is prepared using the Sensititre Yeast Susceptibility inoculum broth and 100 µL of the suspension is inoculated into the dried antifungal containing wells. After inoculation with a standardized suspension of organisms in inoculum medium and

incubation at 35 °C for 24 hours, the minimum inhibitory concentration (MIC) for the test organism is determined by observing the lowest antifungal concentration preventing the development of a pink or purple color change (as evidenced by no color change).

Yeast growth in the antifungal solutions will be evident as change in the colorimetric growth indicator from blue (negative – no growth) to pink/purple (positive – growth). Turbidity is not read, only color change is used as an indicator of growth.

There are several differences between the previously cleared version of the Sensititre YeastOne Susceptibility System with Fluconazole and the current device, including: 1) the Fluconazole dilution range (previously cleared device - 0.125 to 256 µg/mL, current device 0.12 to 128 µg/mL), 2) inoculator (previously cleared device AIM/Autoculator and Manual pipettor, current device AIM/Autoinoculator only), 3) only the VIZION reading method has been used in the current study, and 4) breakpoints have been updated according to the FDA-recognized breakpoints in the STIC website for all indicated *Candida* species.

B Principle of Operation:

Colorimetric test in which MICs are determined by determining the lowest concentration of antifungal agent that shows no color change indicating inhibition of growth of the organism. A color change from blue to pink indicates growth of the organism.

The Sensititre YeastOne panels can be read only with the VIZION viewer which allows the panel image to be displayed on a touch screen directly from a video camera and allows the user to visually determine MIC results.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Sensititre YeastOne Susceptibility System with Voriconazole in the dilution range of 0.008 - 8 ug/mL

B Predicate 510(k) Number(s):
K211539

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Device:** K221198	**Predicate:** K211539
Device Trade Name	Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12 – 128 µg/mL	Sensititre YeastOne Susceptibility System with Voriconazole in the dilution range of 0.008-8 µg/mL
General Device Characteristic Similarities		

Intended Use/Indications For Use	Sensititre YeastOne Susceptibility System is an *in vitro* diagnostic product for clinical susceptibility testing of *Candida* spp.	Same
Organisms Tested	*Candida* spp.	Same
Technology/Instrument	Colorimetric test in which MICs are determined by determining the lowest concentration of antifungal agent that shows no color change indicating inhibition of growth of the organism. A color change from blue to pink indicates growth of the organism.	Same
Test Panel	Each 96 well plate is precision dosed with selected antifungal agents, a stabilizer and the alamarBlue indicator then dried thereby stabilized. The fungal suspension in the appropriate broth is used to rehydrate the plate.	Same
Innoculation Method	AIM autoinoculator	Same
Medium	Sensitire yeast susceptibility inoculum broth.	Same
Incubation Time	24hr	Same
Incubation Temperature	35°C	34 – 36 °C
Reading Method	Vizion	Same
General Device Characteristic Differences		
Antifungal Agent	Fluconazole in the dilution range of 0.12 – 128 µg/mL	Voriconazole in the dilution range of 0.008 – 8 µg/mL
Indicated Species	*Candida albicans* *Candida glabrata* *Candida parapsilosis*	*C. albicans* *C. krusei* *C. parapsilosis*

VI Standards/Guidance Documents Referenced:

CLSI M27-S4. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*; 4th ed. CLSI standard M27 (November 2017).

CLSI M60-S2. *Performance Standards for Antifungal Susceptibility Testing of Yeasts.* 2nd ed. CLSI supplement M60 (June 2020).

FDA Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA (Issued August 28, 2009).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A reproducibility study of the Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12 – 128 µg/mL was performed from 2015-2016. An additional reproducibility study was performed in 2019-2020. Testing was performed at a minimum of 3 sites with isolates tested in triplicate on three separate days. Testing was performed using AIM autoinoculator method and the VIZION reading method only.

The following indicated isolates with known on-scale results were evaluated including *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*.

The overall mode was determined and the reproducibility was calculated based on +/- one dilution and +/- two dilutions of the mode from both reproducibility studies. The best case and worst case reproducibility demonstrated acceptable performance at >90% based on +/- one dilution of the mode. The best case reproducibility was >95% based on +/- 2 dilutions of the mode, which is acceptable (**Table 1**).

Table 1. Reproducibility Analysis for Thermo Scientific Sensititre YeastOne Susceptibility System with Fluconazole

Fluconazole	**Reproducibility based on +/- 1 dilution of the mode**	**Reproducibility based on +/- 2 dilutions of the mode**
Best Case	93.6%	95.1%
Worst Case	90.0%	93.0%

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

Not applicable.

4. Assay Reportable Range:

   Not applicable.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Quality control strains recommended by CLSI were tested with Fluconazole at a minimum of three sites for each clinical study conducted (2015-2016 and 2019-2020). The QC organisms tested were *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.

   Testing was performed daily and was performed a sufficient number of times (at least 20 times/site) using both the Sensititre YeastOne panel and the reference method. Testing was performed using the AIM autoininoculator inoculation method only. The Sensitire YeastOne panels were read using the VIZION and reference panels were read manually using the mirrored reader at 24 hours.

   Results obtained with the two QC strains were acceptable and demonstrated that the panel provides results within the expected range for greater than 95% of tests for each clinical study (**Table 2** and **Table 3**).

Table 2. Results of 2015-2016 QC Testing for the YeastOne with Fluconazole (0.12-128µg/ml).

QC Organisms	Expected Range (Fluconazole, µg/mL)	Concentration (µg/mL)	Sensititre YeastOne (24 hours)	
			Test	Reference
Candida krusei ATCC 6258	8-64	≤0.125		
		0.25		
		0.5		
		1		
		2		
		4		
		8	2	41
		16	0	17
		32	45	2
		64	13	0
		≥128		
Candida parapsilosis ATCC 22019	0.5-4	≤0.125		
		0.25	1	
		0.5	0	0
		1	26	21
		2	33	36
		4	0	3

QC Organisms	Expected Range (Fluconazole, µg/mL)	Concentration (µg/mL)	Sensititre YeastOne (24 hours)	
			Test	Reference
		8		
		16		
		32		
		64		
		≥128		

Table 3. Results of 2019-2020 QC Testing for the YeastOne with Fluconazole (0.12-128µg/ml).

QC Organisms	Expected Range (Fluconazole, µg/mL)	Concentration (µg/mL)	Sensititre YeastOne (24 hours)	
			Test	Reference
Candida krusei ATCC 6258	8-64	≤0.125		
		0.25		
		0.5		
		1		
		2		
		4		
		8	24	0
		16	24	1
		32	30	45
		64	6	38
		≥128		
Candida parapsilosis ATCC 22019	0.5-4	≤0.125		
		0.25		
		0.5	1	0
		1	18	12
		2	47	72
		4	18	0
		8		
		16		
		32		
		64		
		≥128		

Inoculum Density Check:
Colony counts were conducted for all QC and reproducibility isolates, as well as 10% of clinical isolates. All were within the expected range.

Purity Check:
Purity Checks were performed on all isolates following panel inoculation. Only results from pure cultures were evaluated.

Growth Failure:
There were no growth failures on the Sensititre YeastOne Fluconazole panels.

6. Detection Limit:

   Not applicable.

7. Assay Cut-Off:

   Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Clinical testing of Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128 µg/mL was performed in 2015-2016 at two external sites and one internal site and in 2019-2020 at three external sites and one internal site.

   Results obtained with Sensititre YeastOne Susceptibility System with Fluconazole (0.12-128 ug/mL) were compared to results obtained with the CLSI antifungal broth microdilution reference panel with Fluconazole.

   Sensititre YeastOne panels were inoculated using AIM Autoinoculator and results were interpreted using the VIZION only. To address the inoculation and reading methods for the Sensititre YeastOne panels, the following limitation was added in the device labeling:

   > *"Studies of fluconazole with Candida spp. were performed using the AIM autoinoculator inoculation method and the VIZION reading method only. The use of alternative inoculation methods or alternative reading methods when testing fluconazole have not been evaluated."*

   Reference panels were inoculated according to recommendations in the M27 CLSI document and results were interpreted manually using a mirrored reader. The testing conditions for the reference method consisted of the following:

   Media: Roswell Park Memorial Institure (RPMI) 1640 Culture Medium plus 0.2% glucose.
   Inoculum Preparation: per CLSI M27 4th Edition (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts).
   Incubation: 24 hours at 35 °C in ambient air.

   The testing conditions for the Sensititre YeastOne Susceptibility System with Fluconazole (0.012-128 µg/ml) consisted of the following:

   Media: Sensititre Yeast Susceptibility inoculum Broth.

Inoculum Preparation: A standardized suspension (0.5 McFarland) was prepared from a pure 24-hour culture of the yeast isolate in sterile water. Twenty (20) µL of the yeast suspension was inoculated into 11 mL of Yeast Susceptibility Inoculum Broth to create a suspension containing 1.5 to 8.0 X 10^3 CFU/mL. One hundred (100) µL of the broth suspension was inoculated into the plate using the AIM/Autoinoculator.
Incubation: 24 hours at 35°C in ambient air.
Reading: VIZION only.

A total of 735 (572 clinical and 163 challenge) isolates of *Candida* spp were evaluated with both the Sensititre YeastOne Susceptibility panels and the reference panels including 99 *C. albicans* (196 clinical, 59 challenge), 170 *C. glabrata* (119 clinical, 51 challenge), 165 isolates of *C. parapsilosis* (145 clinical, 20 challenge), and 145 *C. tropicalis* (112 clinical, 33 challenge) isolates.

Table 4. Performance of YeastOne Susceptibility System with Fluconazole (0.12-128 µg/mL)

	Tot	# EA	% EA	Eval Tot	# Eval EA	% Eval EA	CA Tot	% CA	# R	# Min	# Maj	# Vmj
***Candida albicans* (Breakpoints (µg/mL): ≤2 S, 4 I, ≥8 R)**												
Clinical	196	192	98.0	159	155	97.5	192	98.0	22	2	1	1
Challenge	59	55	93.2	42	38	90.5	56	94.9	19	2	1	0
Total	255	247	96.9	201	193	96.0	248	97.3	41	4	2	1
***Candida glabrata* (Breakpoints (µg/mL): ≤32 S, ≥64 R)**												
Clinical	119	118	99.2	102	101	99.0	118	99.2	19	1	0	0
Challenge	51	49	96.1	46	44	95.7	51	100.0	11	0	0	0
Total	170	167	98.2	148	145	98.0	169	99.4	32	1	0	0
***Candida parapsilosis* (Breakpoints (µg/mL): ≤2 S, 4 I, ≥8 R)**												
Clinical	145	144	99.3	140	139	99.3	136	93.8	9	10	1	0
Challenge	20	20	100.0	20	20	100	18	90.0	5	2	0	0
Total	165	164	99.4	160	159	99.4	154	93.3	14	12	1	0
***Candida tropicalis* (Breakpoints (µg/mL): ≤2 S, 4 I, ≥8 R)**												
Clinical	112	104	92.9	109	101	92.7	87	77.7	13	21	1	3
Challenge	33	31	93.9	29	27	93.1	29	87.9	2	3	1	0
Total	145	135	93.1	138	128	92.8	116	80.0	15	24	2	3

EA – Essential Agreement min – minor errors
CA – Category Agreement maj – major errors
EVAL – Evaluable Isolates vmj – very major errors
R – Resistant Isolates S – Susceptible Isolates

Essential agreement (EA) occurs when the result of the reference method and that of the Sensititre YeastOne Susceptibility System with Fluconazole are within plus or minus two serial two-fold dilutions of the antibiotic. Evaluable results are those that are on-scale for both the reference method and the Sensititre YeastOne Susceptibility System with Fluconazole. Evaluable results also include results in which an off scale result is more than two doubling dilutions from the on-scale result. Category agreement (CA) occurs when the interpretation of the result of the reference method agrees exactly with the interpretation provided by the Sensititre YeastOne Susceptibility System with Fluconazole.

Performance for the 735 clinical and challenge isolates are shown in **Table 3** and summarized below:

• For *C. albicans*, EA and CA were acceptable, there was one very major error which was considered to be related to incorrect interpretation of the reference method because of trailing endpoints (see below) and was considered a random error.

• For *C. tropicalis*, EA was acceptable however, CA was low at 80.0%. The low CA resulted partly from a high percentage of minor errors (16.5%) and the very major error rate was unacceptable at 20%. The CLSI M27 document indicates that *C. albicans* and *C. tropicalis* have been observed to exhibit significant trailing with the azole antifungals with the broth microdilution reference method and may result in incorrect MIC determination.

To address the low performance for *C. tropicalis*, the sponsor included the following limitation in the device labeling and the organism was removed from the Indications for Use Statement:

"*Due to the low performance of fluconazole with C. tropicalis, isolates of C. tropicalis should be tested with an alternate method.*"

• For *C. albicans*, *C. glabrata, and C. parapsilosis*, the EA and CA were >90% and considered acceptable.

Testing/Reporting MIC for Non-Indicated Species:
For this review, the interpretive criteria are applied to the organisms/organism groups according to the FDA STIC website. As required under 511A(2)(2)(B) of the Federal Food, Drug and Cosmetic Act, the following statement is added to the Precautions section of the device labeling to address testing of non-indicated species:

Per the FDA-Recognized Susceptibility Test Interpretive Criteria website, the safety and efficacy of antimicrobial drugs for which antimicrobial susceptibility is tested by this AST device, may or may not have been established in adequate and well-controlled clinical trials for treating clinical infections due to microorganisms outside of those found in the indications and usage in the drug label. The clinical significance of susceptibility information in those instances is unknown. The approved labelling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.

Resistant Isolates:
A total of 735 clinical and challenge *Candida* spp isolates were tested when the Sensititre YeastOne System with Fluconazole (0.12-128 µg/mL) was compared to the reference method. Each *Candida* spp. was tested with a sufficient number of resistant isolates.

Resistance Mechanisms:
Challenge isolates harboring the resistance mechanisms against Fluconazole which have been shown to be active were not tested. This was addressed in the device labeling by adding the following limitation statement:

"*The performance of the Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128 µg/mL was not evaluated with challenge isolates harboring resistance mechanisms listed in the FDA drug label.*"

Trending:
A trending analysis was conducted using the combined data (clinical and challenge) obtained for Vizion read for each species. This trending calculation takes into account MIC values that are determined to be one or more doubling dilutions lower or higher than the reference method irrespective of whether the device MIC values are on-scale or not. Results that are not clearly at least one dilution lower, at least one dilution higher or in exact agreement with the CLSI reference method are not considered in the trending analysis.

Species for which the difference between the percentage of isolates with higher vs. lower readings was ≥30% and for which the confidence interval was determined to be statistically significant were considered to show evidence of trending. Trending that showed higher or lower MICs values compared to the reference is addressed in the labeling.

Table 5. Trending Observed for *Candida species* tested with the Sensititre YeastOne System with Fluconazole 0.12-128 µg/mL.

Organism	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference[1] (CI)	Trending Noted
C. albicans	242	48 (19.8%)	78 (32.2%)	116 (47.9%)	28.1% (19.8%, 35.8%)	No
C. glabrata	161	42 (26.1%)	40 (24.8%)	76 (47.2%)	21.1% (10.6%, 31.0%)	No
C. parapsilosis	164	35 (21.3%)	72 (43.9%)	57 (34.8%)	13.4% (3.7%, 22.8%)	No
C. tropicalis	140	15 (10.7%)	19 (13.6%)	106 (75.7%)	65.0% (55.1%, 72.6%)	Yes

[1]A positive % difference indicates higher MIC when compared to the reference method.

High trending was observed for *C. tropicalis*. To address the high trending observed with *C. tropicalis*, the following footnote was added to the performance table in the device labeling:

> *"The Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128 µg/mL MIC values tended to be in exact agreement or at least one doubling dilution higher for C. tropicalis compared to the CLSI broth microdilution reference method."*

2. Matrix Comparison:

   Not applicable.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable.

2. Clinical Specificity:

   Not applicable.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable.

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The FDA and CLSI susceptibility interpretive criteria for Fluconazole are as listed in **Table 6**.

Table 6. FDA Recognized Interpretive Criteria for Fluconazole

	Minimum Inhibitory Concentrations (µg/mL)[a]		
Organisms	**S**	**I**	**R**
Candida albicans	≤2	4	≥8
Candida glabrata	-	≤32	≥64
Candida parapsilosis	≤2	4	≥8
Candida tropicalis	≤2	4	≥8

S = Susceptible; I = Intermediate; R = Resistant

[a] FDA-Recognized Antimicrobial Susceptibility Test Interpretive Criteria Website https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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

To support the implementation of changes to FDA-recognized susceptibility test interpretive criteria (i.e., breakpoints), this submission included a breakpoint change protocol that was reviewed and accepted by FDA. This protocol addresses future revisions to device labeling in response to breakpoint changes that are recognized on the FDA STIC webpage (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/ucm410971.htm). The protocol outlined the specific procedures and acceptance criteria that ThermoFisher intends to use to evaluate the Sensititre YeastOne Susceptibility System with Fluconazole in the dilution range of 0.12-128µg/mL when revised breakpoints for Fluconazole are published on the FDA STIC webpage. The breakpoint change protocol included with the submission indicated that if specific criteria are met, ThermoFisher will update the Fluconazole device label to include (1) the new breakpoints, (2) an updated performance section after re-evaluation of data in this premarket notification with the new breakpoints, and (3) any new limitations as determined by their evaluation.