510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231433

B Applicant

Thermo Fisher Scientific

C Proprietary and Established Names

Thermo Scientific Sensititre YeastOne Susceptibility System

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 a substantial equivalence determination for the addition of Rezafungin in the dilution range of 0.008 –8 µg/mL to the YeastOne Susceptibility System for testing *Candida* spp.

B Measurand:

Rezafungin at concentrations of 0.008 –8 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 Rezafungin with new FDA breakpoints and indications for testing Candida spp on the Sensititre YeastOne Susceptibility System.

Rezafungin 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 voriconazole, caspofungin, fluconazole, and rezafungin 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 voriconazole, caspofungin, fluconazole, and rezafungin have not been evaluated.

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

The performance of the Sensititre YeastOne Susceptibility System with Rezafungin in the dilution range of 0.008 - 8 µg/mL was not evaluated with challenge isolates harboring resistance mechanisms listed in the FDA drug label.

D Special Instrument Requirements:

Sensititre AIM for device inoculation
Sensititre VIZION for plate reading

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.

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 digital 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:** K231433	**Predicate:** K211539
Device Trade Name	Sensititre YeastOne Susceptibility System with Rezafungin in the dilution range of 0.008–8 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

Device & Predicate Device(s):	**Device:** K231433	**Predicate:** K211539
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	Sensititre 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	Rezafungin in the dilution range of 0.008 – 8 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 Rezafungin in the dilution range of 0.008 –8 µg/mL was conducted. Testing was performed at 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 two (2) *Candida albicans*, two (2) *Candida glabrata*, two (2) *Candida parapsilosis*, and two (2) *Candida tropicalis*. Additional testing was also performed with (2) *Candida auris,* three (3) *Candida krusei,* one (1) *Candida lusitaniae,* and one (1) *Candida guilliermondii*.

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

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

Rezafungin	Reproducibility based on +/- 1 dilution of the mode	Reproducibility based on +/- 2 dilutions of the mode
Best Case	96.8%	99.8%
Worst Case	93.6%	96.5%

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 Rezafungin at a minimum of three sites. 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. The Sensititre YeastOne panels were inoculated using the AIM autoinoculator and were read using the VIZION. 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**).

Table 2. Results of QC Testing for the YeastOne with Rezafungin (0.008-8µg/ml).

QC Organisms	Expected Range (Rezafungin, µg/mL)	Concentration (µg/mL)	Sensititre YeastOne (24 hours)	Reference (24 hours)
Candida krusei ATCC 6258	0.015-0.12	≤0.008		
		0.15		
		0.03	29	17
		0.06	25	41
		0.12	8	4
		≥0.25		
Candida parapsilosis ATCC 22019	0.25-2	≤0.12		
		0.25	0	2
		0.5	52	35
		1	10	25
		2		
		≥4		

Inoculum Density Check:
Colony counts were conducted for all QC and reproducibility isolates, as well as all 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 Rezafungin 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 Rezafungin in the dilution range of 0.008 - 8 µg/mL was performed at two external sites and one internal site.

   Results obtained with Sensititre YeastOne Susceptibility System with Rezafungin (0.008 - 8 ug/mL) were compared to results obtained with the CLSI antifungal broth microdilution reference panel with Rezafungin.

   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 updated in the device labeling, the updates are shown in bold:

   > *"Studies of voriconazole, caspofungin, fluconazole, **and rezafungin** 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 voriconazole, caspofungin, fluconazole, **and rezafungin** 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 Rezafungin (0.008 - 8 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 334 (254 clinical and 80 challenge) isolates of *Candida* spp were evaluated with both the Sensititre YeastOne Susceptibility panels and the reference panels including 128 *C. albicans* (105 clinical, 23 challenge), 122 *C. glabrata* (78 clinical, 44 challenge), and 84 *C. parapsilosis* (71 clinical, 13 challenge) isolates.

Performance for the 334 clinical and challenge isolates are shown in **Table 3** and summarized below:

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

Table 3. Performance of YeastOne Susceptibility System with Rezafungin (0.008 - 8 µg/mL)

	Tot	# EA	% EA	Eval Tot	# Eval EA	% Eval EA	CA Tot	% CA	# NS	# Min	# Maj	# Vmj
***Candida albicans* (Breakpoints (µg/mL): ≤ 0.12 S)**												
Clinical	105	103	98.1%	87	85	97.7%	102	97.1%	5	N/A	3	0
Challenge	23	22	95.7%	18	17	94.4%	23	100.0%	2	N/A	0	0
Total	128	125	97.7%	105	102	97.1%	125	97.7%	7	N/A	3	0
***Candida glabrata* (Breakpoints (µg/mL): ≤ 0.12 S)**												
Clinical	78	78	100.0%	78	78	100.0%	77	98.7%	3	N/A	1	0
Challenge	44	44	100.0%	44	44	100.0%	42	95.5%	25	N/A	1	1
Total	122	122	100.0%	122	122	100.0%	119	97.5%	28	N/A	2	1
***Candida parapsilosis* (Breakpoints (µg/mL): ≤ 2 S)**												
Clinical	71	71	100.0%	71	71	100.0%	70	98.6%	1	N/A	0	1
Challenge	13	13	100.0%	13	13	100.0%	7	53.8%	6	N/A	0	6
Total	84	84	100.0%	84	84	100.0%	77	91.7%	7	N/A	0	7

EA – Essential Agreement
CA – Category Agreement
EVAL – Evaluable Isolates
NS – Non-susceptible Isolates
min – minor errors
maj – major errors
vmj – very major errors

Essential agreement (EA) occurs when the result of the reference method and that of the Sensititre YeastOne Susceptibility System with Rezafungin 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 Rezafungin. 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 Rezafungin.

Additionally, the sponsor evaluated 82 *C. tropicalis* (62 clinical and 20 challenge) isolates. However, the performance testing with *C. tropicalis* was unacceptable due to poor categorical agreement and a high major error rate (75.6% CA, 20/77 = 26.0% major error rate) as shown in Table 4. After performing an essential agreement adjustment due to the lack of an intermediate category, the adjusted major error rate was still unacceptable (6/77 = 7.8%) and the organism

was removed from the intended use and a limitation statement was added for this organism in the device labeling.

Table 4. Performance of YeastOne Susceptibility System with Rezafungin (0.008 - 8 µg/mL) with *Candida tropicalis*

	Tot	# EA	% EA	Eval Tot	# Eval EA	% Eval EA	CA Tot	% CA	# NS	# Min	# Maj	# Vmj
***Candida tropicalis* (Breakpoints (µg/mL): ≤ 0.12 S)**												
Clinical	62	57	91.9%	61	56	91.8%	45	72.6%	2	N/A	17	0
Challenge	20	18	90.0%	20	18	90.0%	17	85.0%	3	N/A	3	0
Total	82	75	91.5%	81	74	91.4%	62	75.6%	5	N/A	20	0

To address the unacceptable performance when testing *C. tropicalis*, the following limitation was updated in the device labeling (updates are shown in **bold**):

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

In addition, the following footnote was included in the performance table in the device labeling:

> *"% Category Agreements are particularly low for C. tropicalis with rezafungin due to a high incidence of major categorical discrepancies. Please see limitation 10 in the limitations section."*

For *C. parapsilosis,* the potential very major error rate is 100% (7/7). However, due to the lack of an intermediate breakpoint for rezafungin and considering that the observed potential very major errors displayed an essential agreement (i.e., within two doubling dilutions for antifungals), the adjusted potential very major error rate is 0%.

To address the potential very major error rate for *C. parapsilosis*, the following performance footnote was included in the device labeling:

> "*The overall categorical potential very major error rate for Rezafungin when testing C. parapsilosis clinical and challenge isolates is 100% (7/7). Based on the essential agreement (two doubling dilutions for antifungals) and lack of an intermediate breakpoint for Rezafungin, the overall adjusted potential very major error rate for C. parapsilosis clinical and challenge isolates is 0% (0/7).*"

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.

Resistance Mechanisms:
Challenge isolates harboring the resistance mechanisms against Rezafungin 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 Rezafungin in the dilution range of 0.008 - 8 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 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 4. Trending Observed for *Candida* species tested with the Sensititre YeastOne System with Rezafungin 0.008 - 8 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	122	14 (11.5%)	27 (22.1%)	81 (66.4%)	55.0% (43.8%, 63.9%)	Yes
C. glabrata	122	64 (52.3%)	44 (36.1%)	14 (11.5%)	-41.0% (-50.7%, -29.8%)	Yes
C. parapsilosis	84	33 (39.3%)	40 (47.6%)	11 (13.1%)	-26.2% (-38.3%, -13.0%)	No

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

A trend toward higher MIC values was observed for *C. albicans*. To address the observed trending, the following footnote was added to the performance table in the device labeling:

"The Sensititre YeastOne Susceptibility System with Rezafungin in the dilution range of 0.008 - 8 µg/mL MIC values tended to be in exact agreement or at least one doubling dilution higher for C. albicans compared to the CLSI broth microdilution reference method."

A trend toward lower MIC values was observed for *C. glabrata*. To address the observed trending, the following footnote was added to the performance table in the device labeling:

"The Sensititre YeastOne Susceptibility System with Rezafungin in the dilution range of 0.008 - 8 µg/mL MIC values tended to be in exact agreement or at least one doubling

dilution lower for C. glabrata 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 susceptibility interpretive criteria for Rezafungin are as listed in **Table 5**.

Table 5. FDA Recognized Interpretive Criteria for Rezafungin

	Minimum Inhibitory Concentrations (µg/mL)[a]		
Organisms	**S**	**I**	**R**
Candida albicans	≤0.12	-	-
Candida glabrata	≤0.12	-	-
Candida parapsilosis	≤2	-	-
Candida tropicalis	≤0.12	-	-

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 Rezafungin in the dilution range of 0.008-8µg/mL when revised breakpoints for Rezafungin 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 Rezafungin 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.