510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K193024

B Applicant

Thermo Fisher Scientific

C Proprietary and Established Names

Sensititre 20-24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint, Susceptibility System with Lefamulin in the dilution range of 0.008-16 µg/mL

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JWY, LRG, LTT	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 Lefamulin to the Sensititre 20-24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint, Susceptibility System with Lefamulin in the dilution range of 0.008 – 16 ug/mL

B Measurand:

Lefamulin in the dilution range of 0.008 – 16 µg/mL

C Type of Test:

Quantitative Antimicrobial Susceptibility Test (AST), growth-based detection

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Sensititre 20 - 24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint Susceptibility System is an in vitro diagnostic product for clinical susceptibility testing of *H. influenzae, Streptococcus pneumoniae,* and *Streptococcus* spp.

This 510(k) is for Lefamulin in the dilution range of 0.008 - 16 ug/mL for testing *Streptococcus* spp. and *Haemophilus influenzae* on the Sensititre 20 - 24 hour MIC panel.

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

Streptococcus pneumoniae
Haemophilus influenzae

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

The performance of lefamulin with *Haemophilus influenzae* and *Streptococcus pneumoniae* was evaluated using the AIM autoinoculator only. The use of an alternative inoculation system when testing lefamulin has not been evaluated.

The reading of lefamulin results for *S. pneumoniae* was performed using the AutoReader (OptiRead) and VIZION reading methods. The reading of lefamulin results for *Haemophilus influenzae* was only performed by VIZION method. The use of alternative reading methods when testing lefamulin has not been evaluated.

The ability of the Sensititre system to detect resistance to Lefamulin in the following species is unknown because non-susceptible strains were not available at the time of comparative testing: *S. pneumoniae* and *H. influenzae*. Isolates yielding Lefamulin MIC results suggestive of a non-susceptible interpretive category should be submitted to a reference laboratory.

Due to the lack of an intermediate category and observed trending towards higher dilutions for Lefamulin, testing of *H. influenzae* have resulted in major discrepancies for isolates that are otherwise within essential agreement for the reference method. Testing should be repeated using an alternative testing/reference method prior to reporting results for *H. influenzae* when the Sensititre with the VIZION is 4 μg/mL.

D Special Instrument Requirements:

Sensititre AIM for device inoculation

Sensititre VIZION or OptiRead for plate reading (results for *Haemophilus influenzae* are interpreted using VIZION only)

IV Device/System Characteristics:

A Device Description:

Sensititre MIC Susceptibility MIC panels are multi-well microtiter plates, dosed with dried, stabilized antimicrobials. It is a miniaturized version of the classic broth dilution method and can provide both qualitative and quantitative susceptibility results. After inoculation, plates are sealed with an adhesive seal, incubated at 34 – 36 °C for 20 – 24 hours and examined for bacterial growth.

Antimicrobial susceptibility test results can be determined by reading growth using the digital viewing device (VIZION) or automatically on an autoreader (OptiRead) using fluorescence.

B Principle of Operation:

The Sensititre 20 - 24 hour *Haemophilus influenzae/Streptococcus pneumoniae* system includes multi-well plastic microtiter plates that contain doubled dilutions of antibacterial agents. Each plate includes antimicrobial agents at appropriate dilutions. Results can be read by the digital device, VIZION, or by use of an automated reader, OptiRead (*Streptococcus* spp. only).

The VIZION 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. The Sensititre OptiRead utilizes fluorescence technology to read the microbroth dilution plates after 20 to 24 hours incubation. The technology involves the detection of bacterial growth by monitoring the activity of specific surface enzymes produced by the test organism. Growth is determined by generating a fluorescent product from a fluorogenic substrate. The substrate is prepared by conjugating a fluorescent compound to the specific enzyme substrates with a bond which prevents fluorescence. The enzymatic action of the bacterial surface enzymes on the substrate cleaves the bond releasing fluorescence. The amount of fluorescence detected is directly related to bacterial growth. The MIC is determined by observing the lowest dilution of antimicrobial agent that inhibits growth of the organism. The substrate can be added to the inoculum broth which is dispensed into the test plate at the same time as the test organism, or, the plates can be prepared with the substrate already added to each micro-well.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Sensititre 20-24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint Susceptibility System with Omadacycline in the dilution range of 0.008 - 32 ug/mL

B Predicate 510(k) Number(s):

K183324

C Comparison with Predicate(s):

Table 1: Comparison with the Predicate

Device & Predicate Device(s):	**Device** **K193024**	**Predicate** **K183324**
Device Trade Name	**Sensititre *Haemophilus/Streptococcus pneumoniae* (HP) MIC Plates with Lefamulin**	**Sensititre *Haemophilus/Streptococcus pneumoniae* (HP) MIC Plates with Omadacycline**
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Sensititre *Haemophilus influenzae/Streptococcus pneumoniae* plates are in vitro diagnostic products for clinical susceptibility testing of *Haemophilus influenzae, Streptococcus pneumoniae,* and *Streptococcus* species.	Same
Test Panel	96 well plate is dosed with selected antimicrobial agents and substrate for the fluorescent reads, then dried. The bacterial suspension in the appropriate broth is used to rehydrate the plate.	Same
Test Organism	*Haemophilus influenzae, Streptococcus pneumoniae,* and *Streptococcus spp.*	Same
Reading Methods for *Streptococcus* spp.	Results can be read using the following methods: 1) Automatically with the OptiRead (fluorescent substrate technology) 2) On the VIZION (digital viewing device)	Same
Reading Methods for *Haemophilus*	Results can be read using the VIZION (digital viewing device) only	Same
Incubation	20-24 hours, 35 ± 1° C	Same
Inoculation Media	CAMHBT + LHB (*Streptococcus* spp.), HTM broth (*H. influenzae*)	Same
General Device Characteristic Differences		
Antimicrobial Agent	Lefamulin	Omadacycline

Concentration Range	0.008 – 16 µg/mL	0.008 – 32 µg/mL

VI Standards/Guidance Documents Referenced:

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

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.

CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   A reproducibility study was performed at four sites using a panel comprised of 10 isolates of *Streptococcus* spp.: *S. pneumoniae* (three isolates), *S. pyogenes* (one isolate), *S. agalactiae* (two isolates), *S. anginosus* (two isolates), *S. mitis* (one isolate), and *S. salivarius* (one isolate) and a panel comprised of 10 strains of *H. influenzae*. Of the *Streptococcus* spp., five non-indicated species (7 isolates) were tested but this was considered acceptable due to similarities among the species and all isolates provided MIC values that are on-scale. All isolates were tested in triplicate over three days with each read method (i.e., VIZION and OptiRead for *Streptococcus* spp., and VIZION only for *H. influenzae*). The Sensititre AIM inoculator was used for plate inoculation. The mode MIC value was determined and the reproducibility was calculated based on MIC values falling within ±1 dilution of the mode MIC value. Reproducibility was greater than 95% for both read methods for *Streptococcus* spp. and VIZION for the *H. influenzae* panel; results were considered to be acceptable.

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 controls strains recommended by CLSI were tested with Lefamulin at four sites. The QC organisms tested were *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247. The QC strains were tested a minimum of 20 times per site and read using the OptiRead (*S. pneumoniae* only) and VIZION (both *S. pneumoniae* and *Haemophilus influenzae*). The results demonstrate that the Sensititre Lefamulin MIC quality controls results were within the recommended range ≥95% of the time (Table 2).

Table 2. Quality Control Results for Sensititre 18 – 24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint Susceptibility System with Lefamulin with the VIZION and OptiRead Methods.

QC Organism	Lefamulin Range (µg/mL)	Concentration (µg/mL)	Reference	Read Method	
				VIZION	OptiRead*
S. pneumoniae ATCC 49619	0.06 – 0.5	0.03	0	0	0
		0.06	12	4	6
		0.12	40	37	50
		0.25	36	50	35
		0.5	0	0	0
		1	0	0	0
H. influenzae ATCC 49247	0.5 - 2	0.25	1	0	N/A
		0.5	12	10	N/A
		1	66	43	N/A
		2	0	34	N/A
		4	0	0	N/A

*QC results for *H. influenzae* were interpreted only using the VIZION.

Inoculum Density. Inoculum density checks were performed a sufficient number of times; all organism suspensions were in the acceptable range.

Purity checks. Purity checks were performed on all isolates following plate inoculation. Only results from pure cultures were evaluated.

Growth Failures. All isolates tested showed growth in the Sensititre panels.

6. Detection Limit:

   Not applicable

7. Assay Cut-Off:

   Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

Results obtained with Sensititre 20 - 24 hour *Haemophilus influenzae/Streptococcus pneumoniae* MIC or Breakpoint Susceptibility System with Lefamulin were compared to results obtained with the CLSI broth microdilution reference panel. Drug dilutions were prepared using fresh Mueller Hinton broth with lysed horse blood (LHB) for *Streptococcus* spp. and Haemophilus Test Medium (HTM) broth for *H. influenzae* isolates as indicated in CLSI M07, 11th ed. Clinical testing was performed at four sites, three of which were in the U.S. A total of 200 clinical *S. pneumoniae* isolates were tested and read by both VIZION and OptiRead. In addition, a total of 393 *H. influenzae* isolates were tested of which results were read using the digital viewing device (VIZION) only. All of the clinical isolates tested were contemporary isolates (i.e., tested within 6 months of isolates). During the course of the clinical trial, all Sensititre dried MIC panels were inoculated using the Sensititre Autoinoculator (AIM) and the same panel was read on both the VIZION and the OptiRead for *S. pneumoniae* isolates in a blinded manner. The sponsor added the following limitations to the device labeling to reflect these inoculation and read methods:

> *The performance of lefamulin with Haemophilus influenzae and Streptococcus pneumoniae was evaluated using the AIM autoinoculator only. The use of an alternative inoculation system when testing lefamulin has not been evaluated.*
>
> *The reading of lefamulin results for S. pneumoniae was performed using the AutoReader (OptiRead) and VIZION reading methods. The reading of lefamulin results for Haemophilus influenzae was only performed by VIZION method. The use of alternative reading methods when testing lefamulin has not been evaluated.*

A total of 100 challenge isolates were tested at a single site. Species tested included *S. pneumoniae* (50 isolates) and *H. influenzae* (50 isolates).

Results for *S. pneumoniae* and *H. influenzae* were evaluated for essential agreement (EA) and category agreement (CA) when compared to the reference method. Breakpoints for both are noted on the FDA-Recognized Susceptibility Test Interpretive Criteria Website (STIC). Essential Agreement (EA) is when the Sensititre results agree exactly or within one doubling dilution of the reference broth microdilution results. Category Agreement (CA) is when the Sensititre result interpretation agrees exactly with the reference broth microdilution result interpretation.

The results from clinical and challenge testing determined with the VIZION demonstrated a combined EA of 99.2% and CA of 98.4% for *S. pneumoniae* (Table 3). There was one major error (1/245 =0.4%) and 3 very major errors (3/5 = 60%). Due to the lack of an intermediate interpretive category, our analysis takes into consideration MIC results that are within essential agreement and the error rate is adjusted accordingly. For lefamulin, the MIC values for all four of these categorical errors were within essential agreement of the reference method. The adjusted error rate is 0% for major errors and 0% for very major errors. When interpreted by the OptiRead (Table 4), results demonstrated a combined EA of 98.8% and CA of 98.8%. No major errors were identified, however, the same three very major errors were identified when interpreted by the OptiRead. Similarly as with the VIZION, because all three very major errors were within essential agreement, the adjusted very major error rate is 0% and therefore, acceptable.

The results from clinical and challenge testing determined with the VIZION demonstrated a combined EA of 98.9% and CA of 94.8% for *H. influenzae* (Table 3). There were 23 (23/429=5.4%) major errors and no very major errors identified. Due to the lack of an intermediate or resistant interpretive category, our analysis takes into consideration MIC results that are within essential agreement and the error rate is adjusted accordingly. For lefamulin, many of the observed major errors were otherwise within essential agreement of the reference method. The adjusted major error rate was 0.2% (1/429). However, given the high number of major errors and trending in favor of higher dilutions when compared to the reference method (see Table 5), the following was included in the labeling to instruct users to test with an alternative method if a lefamulin MIC result of 4 µg/mL is obtained:

Due to the lack of an intermediate category and observed trending towards higher dilutions for Lefamulin, testing of H. influenzae have resulted in major discrepancies for isolates that are otherwise within essential agreement for the reference method. Testing should be repeated using an alternative testing/reference method prior to reporting results for H. influenzae when the Sensititre with the VIZION is 4 µg/mL.

Table 3. Performance of *S. pneumoniae* and *H. influenzae* Clinical and Challenge Isolates, Read Using VIZION

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. NS	No. S	min	maj	vmj
S. pneumoniae													
Clinical	200	198	99.0	200	198	99.0	198	99.0	4	196	NA	0	2
Challenge	50	50	100	50	50	100	48	96.0	1	49	NA	1	1
Total	250	248	99.2	250	248	99.2	246	98.4	5	245	NA	1	3[1]
H. influenzae													
Clinical	393	388	98.7	386	381	98.7	372	97.7	13	380	NA	21	0
Challenge	50	50	100	50	50	100	48	96.0	1	49	NA	2	0
Total	443	438	98.9	436	431	98.9	420	94.8	14	429	NA	23[2]	0

[1]Adjusted very major error rate is 0%.
[2]Adjusted major error rate is 0.2% (1/429).

Table 4. Performance of *S. pneumoniae* Clinical and Challenge Isolates, Read Using OptiRead

	Tot	EA N	EA %	Eval Tot	Eval EA N	Eval EA %	CA Tot	CA %	No. NS	No. S	min	maj	vmj
S. pneumoniae													
Clinical	200	197	98.5	200	197	98.5	198	99.0	4	196	NA	0	2
Challenge	50	50	100	50	50	100	49	98.0	1	49	NA	0	1
Total	250	247	98.8	250	247	98.8	247	98.8	5	245	NA	0	3

[1]Adjusted very major error rate is 0%.

EA – Essential agreement
CA – Category agreement
Eval – Evaluable isolates
NS – Non-susceptible isolates
NA – Not Applicable due to lack of intermediate category
maj – Major errors
vmj – Very major errors
min – Minor errors
S - Susceptible

To address the testing and reporting of non-indicated species the following comment was added to the Precautions section of the device labeling:

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.

Due to the lack of intermediate or resistant categories and availability of insufficient number of non-susceptible isolates tested in the comparison study, the sponsor also included the following to instruct users to use an alternative method of testing when non-susceptible isolates of *S. pneumoniae* and *H. influenzae* are encountered:

The ability of the Sensititre system to detect resistance to Lefamulin in the following species is unknown because non-susceptible strains were not available at the time of comparative testing: S. pneumoniae and H. influenzae. Isolates yielding Lefamulin MIC results suggestive of a non-susceptible interpretive category should be submitted to a reference laboratory.

MIC Trending

An analysis of trending was calculated using the combined clinical and challenge data for each organism group. This trending calculation takes into account MIC values that are determined to be one or more doubling dilutions lower or higher compared to the reference method irrespective of whether the device MIC values are on-scale or not. Trending results are shown in Table 5. Results for *S. pneumoniae* were stratified by both reading methods and *H. influenzae* was evaluated using the VIZION only to determine if particular trends were observed. The acceptable percent difference between higher and lower dilution readings is <30%. Based on the results observed, a trend was identified for *H. influenzae* when interpreted by the VIZION which trended towards higher dilutions when compared to the reference method. Given this, the following was included in the package insert as a footnote:

Lefamulin MIC values tended to be in exact agreement or at least one doubling dilution higher when testing H. influenzae with the VIZION reading method compared to the CLSI reference broth microdilution.

Table 5: Lefamulin Trending Analysis for *S. pneumoniae* and *H. influenzae*, Read by VIZION and OptiRead (*S. pneumoniae* only)

Organism (Read Method)	Total Evaluable for Trending	≥ 1 Dilution lower No. (%)	Exact No. (%)	≥ 1 Dilution Higher No. (%)	Percent Difference (CI)	Trending Noted
***S. pneumoniae* (VIZION)**	250	67 (26.8)	157 (62.8)	26 (10.4)	-16.4 (-23.0 to -9.7)	No
***S. pneumoniae* (OptiRead)**	250	80 (32.0)	154 (61.6)	16 (6.4)	-25.6 (-32.1 to -19.0)	No
***H. influenzae* (VIZION)**	438	10 (2.3)	191 (43.6)	237 (54.1)	51.8 (46.8 – 56.6)	Yes

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-identified susceptibility interpretive criteria for lefamulin are as listed in Table 6.

Table 6: FDA-Recognized Interpretive Criteria[a] for Lefamulin (µg/mL)

	Susceptible (S)	Intermediate (I)	Resistant (R)
Streptococcus pneumoniae	≤0.5	-	-
Haemophilus influenzae	≤2	-	-

[a] FDA STIC Webpage

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 20-24 hour *Haemophilus influenzae/Streptococcus*

pneumoniae MIC or Breakpoint, Susceptibility System with Lefamulin in the dilution range of 0.008-16 ug/ml when revised breakpoints for lefamulin 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 lefamulin 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.