



Final Report

Protocol Title: **Validation of a Disinfection Procedure for the Konica Minolta 9VE4 4D EC Transducer Supplied by Siemens Healthineers with the Trophon® 2 System**

Protocol #: 19-L115FR

LexaMed, Ltd.

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Originator: Erin Huber

Date: 4/22/2020

LexaMed Approvals

Study Director: Erin Huber Date: 4/22/20
Printed Name Erin C. Huber Signature

Technical Review: Anne Schuler Date: 4/22/20
Printed Name Anne M. Schuler Signature

Quality Assurance: Brandi Heckman Date: 4/22/20
Printed Name Brandi R. Heckman Signature

1. Summary

- 1.1. The purpose of this study was to validate the Trophon® 2 as an automated high level disinfection system for the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers.
- 1.2. Testing was conducted using the guidance documents listed below. The protocol was initiated on February 10, 2020 and completed on February 27, 2020.
 - 1.2.1. FDA guidance document: *Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling* (March 2015)
 - 1.2.2. AAMI TIR 12 – *Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for medical device manufacturers*.
 - 1.2.3. ANSI/AAMI/ISO 17664:2017 – *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*.
- 1.3. The 9VE4 4D EC Transducer was inoculated with a *Mycobacterium terrae* microorganism suspension. The efficacy of the automated disinfection process was evaluated based on residual levels of the microorganism marker.
- 1.4. The residual *Mycobacterium terrae* microorganism results met the acceptance criteria for Trophon® 2 system disinfectant efficacy cycles conducted on the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers.
- 1.5. Based on the results of this study, the procedure as defined in this report using Sonex-HL® solution within the Trophon® system was shown to be an effective High Level Disinfectant for the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers.

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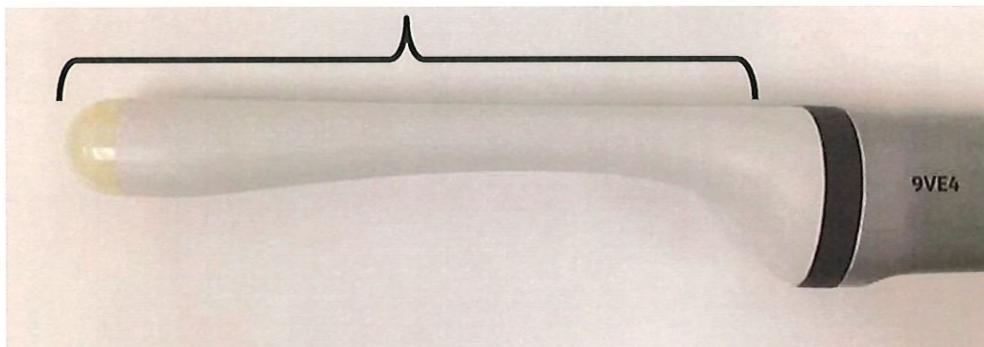
2. Sample Identification

- 2.1. 9VE4 4D EC Transducer, P/N: 11289564

Figure 1: 9VE4 4D EC Transducer



Figure 2: 9VE4 4D EC Transducer Test Area



- 2.1.1. Only the patient contact portion of the probe as shown in Figure 2 was tested. The handle portion was not evaluated in this study.

- 2.2. The Sonex® HL cartridges (P/N: N05002), lot number C80901 (exp. Sept. 24, 2020) were used as the disinfecting agent for the Trophon® 2 automated high level disinfection system. The cartridges contained hydrogen peroxide (H_2O_2) at a concentration of 34-35%.

3. Procedure

- 3.1. Preliminary Cleaning

- 3.1.1. The preliminary cleaning process was conducted on each of the test samples prior to initiating the study.

NOTE: This cleaning procedure was intended ONLY for laboratory use.

- 3.1.2. A small identification was marked on each component to ensure traceability throughout the protocol activities.

- 3.1.3. Gloves were worn in the detergent solution preparation and cleaning procedures.

- 3.1.4. An ENZOL® Enzymatic Detergent solution was prepared in a container by diluting one (1) ounce (29.6 mL) of ENZOL® Enzymatic Detergent per gallon (3785 mL) of tap water at a temperature of 20-25°C. Temperatures were verified with a calibrated thermometer.

- 3.1.5. Test components were cleaned as a batch.

- 3.1.6. Test components were submerged as a batch in the prepared solution for five (5) minutes.



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- 3.1.7. After five (5) minutes, the test components were removed from the detergent solution and wiped with a non-linting wipe.
 - 3.1.8. Once all test components were wiped, they were rinsed individually under running tap water at 20-25 °C for a minimum of thirty (30) seconds.
 - 3.1.9. Immediately following the tap water rinse, a secondary rinse in DI water was performed by submerging the components into one gallon (3,785 mL) of DI water for one (1) minute.
 - 3.1.10. The test components were wiped dry with a clean, dry wiper, and allowed to completely dry.
- 3.2. Validated Microbial Recovery Method
- 3.2.1. A recovery method was previously validated for this transducer under LexaMed protocol 19-L114. The validated method is outlined below:
 - 3.2.1.1. The test articles were placed into a sterile plastic bag and covered with 500mL sterile Peptone Recovery Buffer.
 - 3.2.1.2. The sterile bags were placed on a rotary shaker set at approximately 200 rpm for twenty (20) minutes.
 - 3.2.1.3. Based on the results from the previous method validation a recovery factor (RF) of 1.04 was established using this method. This RF was applied to disinfection efficacy results for this study.
- 3.3. Verification of Disinfectant Neutralization
- 3.3.1. Prior to testing, all test articles intended for the neutralization study were cleaned per section 3.1 then wiped with a non-linting cloth dampened with sterile isopropyl alcohol (IPA) and allowed to dry.
 - 3.3.2. Sufficient neutralization to allow for the enumeration of *Mycobacterium terrae*, was verified for the Sonex HL disinfectant solution within the Trophon® 2 system. Neutralization test procedures were conducted within a LAF hood.
 - 3.3.3. Three (3) test article replicates were utilized for the disinfectant neutralization procedure.
 - 3.3.4. Disinfection
 - 3.3.4.1. Each of the three (3) test articles were disinfected as follows:
 - 3.3.4.1.1. Devices were individually disinfected in the Trophon® 2 System using the manufacturer provided Sonex® HL cartridges with hydrogen peroxide at the manufacturers acceptable high end concentration of 36-37%.
 - 3.3.4.1.2. A fresh pair of gloves were worn by the operating technician for loading and post-disinfection removal of the device.
 - 3.3.4.1.3. A non-linting wipe was used to ensure the device was completely dry immediately prior to loading into the Trophon®.
 - 3.3.4.1.4. The device was loaded into the system's disinfection chamber, ensuring it did not come into contact with the chamber walls, and was situated above the embossed line. See Figure 3 for proper disinfection loading orientation of the 9VE4 4D EC Transducer.

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Figure 3: 9VE4 4D EC Trophon® 2 Loading Orientation



- 3.3.4.1.5. A Trophon® chemical indicator was placed, red side up, into the locator at the base of the chamber door.
- 3.3.4.1.6. The chamber door was closed and the start button was pressed to initiate the seven (7) minute disinfection cycle.
- 3.3.4.1.7. At the completion of the disinfection cycle, the operating technician verified the chemical indicator color change against the manufacturers color assessment chart on the chemical indicator carton.
- 3.3.4.1.8. The device was removed with gloved hands, wiped with a clean, non-linting wipe, and immediately placed into an extraction vessel.
- 3.3.4.1.9. The chamber door was closed and the device transferred to a LAF hood for testing.

3.3.5. Extraction

- 3.3.5.1. The disinfected components were extracted using the microbial recovery method as stated in section 3.2.

3.3.6. Neutralization Efficacy

- 3.3.6.1. A working suspension of *Mycobacterium terrae* was prepared in a 5% Fetal Bovine Serum (FBS) / Lethen Broth (LB) to target approximately 100 CFU/0.1 mL.
- 3.3.6.2. Extraction containers were agitated to mix at the time of filtration.
- 3.3.6.3. The extraction fluid was directly inoculated with 0.1 mL of the microbial suspensions prepared in section 3.3.6.1. The fluid from each extraction was separately filtered through a 0.45 µm filter. Filters were rinsed three (3) separate times with 50 mL of Lethen Broth (LB). Filters were then plated as follows:
 - 3.3.6.3.1. The filters were transferred to individual pre-poured M7H10 with T80 and lecithin agar plates that were supplemented with Middlebrook OADC



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enrichment broth (approximately 1 mL Middlebrook OADC enrichment broth per 9 mL M7H10 agar). One (1) negative control was similarly prepared and plated.

3.3.6.4. Toxicity Control Plates

- 3.3.6.4.1. Toxicity control plates were prepared to verify that there was no negative impact of the disinfectant efficacy neutralizer solution on the population of the challenge organism.
- 3.3.6.4.2. Three (3) identical aliquots of the sterile peptone utilized for the extraction procedures were transferred into individual sterile containers. The samples were similarly filtered, inoculated, and plated as the extract solutions described in section 3.3.6.3 utilizing the Lethen Broth neutralizer rinses.

3.3.6.5. Viability Controls

- 3.3.6.5.1. An inoculum verification of the microbial suspension prepared in section 3.3.6.1 was conducted in triplicate by inoculation of the suspension into a 100 mL aliquot of peptone then filtering. Filters were plated as described in sections 3.3.6.3.1.

- 3.3.7. Plates were incubated at 34-38°C for 7-14 days or until colonies were readily visualized.
- 3.3.8. Following incubation, all plates were enumerated, results recorded, and the percent (%) recovery determined by comparing the Neutralizer Efficacy plate counts and the Toxicity Control plate counts with those obtained from the Viability Control plate counts.
- 3.3.9. The following equation was used to calculate the Neutralizer Efficacy and the Toxicity percent recoveries:

$$\frac{\text{Average Recoverable CFU (average of efficacy replicates OR average of toxicity control replicates)}}{\text{Average Recoverable CFU (average of viability control replicates)}} \times 100\%$$

3.4. Disinfection Efficacy Testing

- 3.4.1. Prior to disinfection efficacy testing, all test articles intended for disinfection efficacy testing were cleaned per section 3.1 then wiped with a non-linting cloth dampened with sterile isopropyl alcohol (IPA) and allowed to dry in a LAF hood.
- 3.4.2. Three (3) complete disinfection efficacy determination cycles were conducted.
- 3.4.3. Each cycle consisted of microbial inoculation of the device surface, disinfection, and disinfection efficacy determination as follows. Testing was conducted within a LAF hood.

3.4.4. Inoculation Procedure

- 3.4.4.1. A suspension of *M. terrae* was prepared in a 5% FBS/LB solution to achieve a target total microbial population of approximately 10^7 CFU/0.1mL.
- 3.4.4.2. Within a LAF hood, four (4) test articles were inoculated with a total of 0.1 mL of the prepared microbial suspension prepared in section 3.4.4.1 at the locations outlined in Figure 4. A population verification of the microbial suspension was conducted via standard dilution and plating per LEXLP-054, "Standard Plate Count for Total Bacteria and Total Fungi in Liquid and Water Soluble Products".
- 3.4.4.3. The inoculated surfaces were allowed to dry for ten (10) minutes.
- 3.4.4.4. One (1) test article remained un-inoculated to serve as a negative control.

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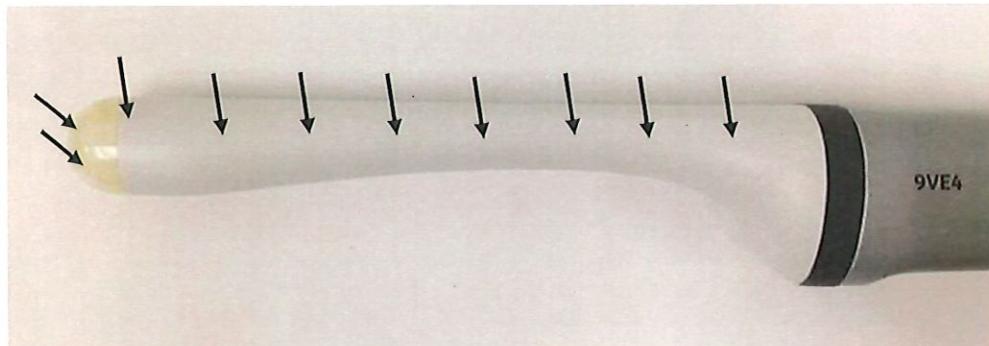
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Figure 4: 9VE4 4D EC Transducer Inoculation Sites



3.4.5. Disinfection Procedure

- 3.4.5.1.** Each of the three (3) inoculated test articles and one (1) un-inoculated negative control test article were disinfected as follows:
 - 3.4.5.1.1.** Devices were individually disinfected in the Trophon® 2 System using the manufacturer provided Sonex® HL cartridges with a concentration of 34-35% hydrogen peroxide.
 - 3.4.5.1.2.** A fresh pair of gloves were worn by the operating technician for loading and post-disinfection removal of the device.
 - 3.4.5.1.3.** A non-linting wipe was used to ensure the device was completely dry immediately prior to loading into the Trophon®.
 - 3.4.5.1.4.** The device was loaded into the system's disinfection chamber, ensuring it did not come into contact with the chamber walls, and was situated above the embossed line. See Figure 3 for proper disinfection loading orientation of the 9VE4 4D EC Transducer.
 - 3.4.5.1.5.** A Trophon® chemical indicator was placed, red side up, into the locator at the base of the chamber door.
 - 3.4.5.1.6.** The chamber door was closed and the start button was pressed to initiate the seven (7) minute disinfection cycle.
 - 3.4.5.1.7.** At the completion of the disinfection cycle, the operating technician verified the chemical indicator color change against the manufacturers color assessment chart on the chemical indicator carton.
 - 3.4.5.1.8.** The device was removed with gloved hands, wiped with a clean, non-linting wipe, and immediately placed into an extraction vessel.
 - 3.4.5.1.9.** The chamber door was closed and the device transferred to a LAF hood for testing.
- 3.4.5.2.** One (1) inoculated test article was not disinfected and was utilized as the positive control.

3.4.6. Disinfection Efficacy Determination

- 3.4.6.1.** The disinfected test articles and negative control and the non-disinfected positive control component were transferred to extraction vessels and extracted using the method as described in section 3.2.
- 3.4.6.2.** The entire extraction volume from the three (3) replicates of the inoculated and disinfected test articles and one (1) replicate of the non-inoculated and disinfected negative control were filtered, rinsed, and directly plated onto M7H10 with T80 and



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lecithin plates supplemented with Middlebrook OADC enrichment broth (approximately 1 mL Middlebrook OADC enrichment broth per 9 mL M7H10 agar).

- 3.4.6.3. The extract from the positive control was serially diluted as required to determine the population. Duplicate 1 mL aliquots from designated dilutions were similarly filtered, rinsed, and plated as described in section 3.4.6.2.
- 3.4.6.4. Plates were incubated at 34-38°C for 7-14 days or until colonies were readily visualized.

3.5. Cytotoxicity Testing

- 3.5.1. Cytotoxicity testing of a disinfected 9VE4 4D EC Transducer was conducted to assess the presence of residual Sonex® HL disinfectant solution from the test component. Testing was conducted per LexaMed SOP LEXLP-047, "Cytotoxicity Elution Test – USP and ISO Methods".

4. Results

- 4.1. The preliminary cleaning was successfully conducted prior to use and following testing.

4.2. Disinfectant Neutralization Verification

- 4.2.1. Disinfectant neutralization was conducted to determine that there was no negative impact from the disinfectant or neutralizer solution on the microbial challenges. A $\geq 70\%$ recovery of *M. terrae* from both the component neutralization verification and toxicity controls was achieved using Lethen Broth. The results are outlined in Table 1. The negative control performed as expected.

Table 1: Disinfectant Neutralization Results

Disinfectant	Neutralization Media	IV Average CFU	Test Article Average CFU	Test Article Avg vs. IV Average	Toxicity Average CFU	Toxicity Avg vs. IV Average	Pass/Fail
Sonex® HL	Lethen Broth	72	67	93%	71	99%	Pass

4.3. Disinfection Efficacy Determination

- 4.3.1. The protocol stated acceptance criteria of a ≥ 6 log reduction was achieved for the Sonex-HL® within the Trophon® 2 system. Results are summarized in Tables 2 – 4.

Table 2: Disinfection Efficacy Inoculation Verification Results

Disinfectant Type	Cycle #	Dilution	Volume Filtered	CFU Counts		Mean CFU	Inoculated Population
				#1	#2		
Sonex-HL®	1	10^{-6}	1 mL	18	37	28	2.8×10^6 CFU/0.1mL
	2 & 3	10^{-7}	1 mL	32	28	30	3.0×10^7 CFU/0.1mL



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Table 3: Disinfection Efficacy Positive Control (PC) Results

Disinfectant Type	Cycle	Dilution of Extract Tested	Volume Filtered (mL)	Actual Recovered CFU		Actual Recovered Population	Extraction Volume	RF	Population ¹	Log of PC Population
Sonex-HL®	1	10 ⁻²	1 mL	25	30	2.8 x 10 ³ CFU	500mL	1.04	1.5 x 10 ⁶ CFU	6.2
	2	10 ⁻²	1 mL	56	39	4.8 x 10 ⁵ CFU			2.5 x 10 ⁷ CFU	7.4
	3	10 ⁻²	1 mL	40	43	4.2 x 10 ⁴ CFU			2.2 x 10 ⁷ CFU	7.3

$$^1 \text{Population} = \left(\frac{\text{Recovered Population CFU}}{\text{Volume Filtered}} \right) \times \left(\frac{500 \text{ mL (Extraction Volume)}}{} \right) \times \text{RF}$$

Table 4: Sonex-HL® Disinfection Efficacy Results

Cycle	Component ID	Volume Filtered (mL)	Recovered CFU	RF to Apply	Corrected Population CFU ¹	Log of Recovered CFU	Log of Positive Control Population	Log Reduction Achieved ²	Met Protocol Requirement of ≥ 6.0 log reduction
1	1	500 mL	1	1.04	1	0	6.2	6.2	Yes
	2		0		< 1	0		6.2	Yes
	3		1		1	0		6.2	Yes
	5 (NC)		0		0				
2	1	500 mL	0	1.04	< 1	0	7.4	7.4	Yes
	2		0		< 1	0		7.4	Yes
	3		0		< 1	0		7.4	Yes
	5 (NC)		0		0				
3	1	500 mL	1	1.04	1	0	7.3	7.3	Yes
	2		3		3	0.5		6.8	Yes
	3		0		< 1	0		7.3	Yes
	5 (NC)		0		0				

$$^1 \text{Corrected Population CFU} = \left(\frac{\text{Total Recovered CFU}}{\# \text{replicates}} \right) \times \left(\frac{500 \text{ mL (Total Extract Volume)}}{\text{Volume Filtered}} \right) \times \text{RF}$$

² Log Reduction Calculation = Log of Positive Control Component – Log of Corrected Population

4.4. Cytotoxicity Test Results

- 4.4.1. The 9VE4 4D EC Transducer disinfected with Sonex® HL solution was non-cytotoxic. A copy of the final report is provided in Attachment 2.

5. Deviations

- 5.1. No deviations occurred during the execution of protocol 19-L115.

6. Conclusion

- 6.1. The automated Trophon® 2, IFU defined disinfection procedure using Sonex® HL solution as defined below produced an overall ≥ 6 log reduction of *Mycobacterium* validating it as a High Level Disinfectant (HLD) for the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers.

6.1.1. Turn the power to the Trophon® 2 System on.

6.1.2. While wearing gloves, use a non-linting wipe to ensure the probe is completely dry prior to loading.



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- 6.1.3. Load the probe into the systems disinfectant chamber, situating above the embossed line.
- 6.1.4. Place a Trophon® chemical indicator, red side up, into the locator at the base of the chamber door.
- 6.1.5. Close the chamber door and press the 'Start' button to initiate the disinfection cycle.
- 6.1.6. While wearing a new set of gloves, remove the probe and wipe with a clean, non-linting wipe and store in a clean probe cover.
- 6.1.7. Remove the chemical indicator to verify color change.

7. References

- 7.1. AAMI TIR 12 – *Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for manufacturers – 3rd edition.*
- 7.2. Guidance for Industry and Food and Drug Administration Staff: *Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling* (March 2015).
- 7.3. ANSI/AAMI/ISO 17664:2017 – *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*
- 7.4. LexaMed SOP LEXLP-054, "Standard Plate Count for Total Bacteria and Total Fungi in Liquid and Water Soluble Products".
- 7.5. SOP LEXLP-047, "MEM Elution Cytotoxicity Test – USP and ISO Methods".
- 7.6. LexaMed protocol 19-L114, "Validation of Recovery Extraction Methods for use in Cleaning and High Level Disinfectant Validation Studies for the Konica Minolta 9VE4 4D EC Transducer Supplied by Siemens Healthineers".

8. Record Storage

- 8.1. All raw data pertaining to this study as well as the final report will be maintained in the LexaMed archives for a minimum of fifteen (15) years.

9. Attachments

- 9.1. Attachment 1: Personnel Identification
- 9.2. Attachment 2: Cytotoxicity Final Report
- 9.3. Attachment 3: Execution Copy of Protocol 19-L115



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ATTACHMENT 1 PERSONNEL IDENTIFICATION



Protocol

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ATTACHMENT 1

Each person who will be recording data on any worksheet included in this protocol must complete an entry on this page. Signatures signify they have read the protocol and understand the objectives, acceptance criteria, and procedural requirements defined prior to protocol execution.

Reviewed by: Kim Munn

Date: 4/16/16

* Technicians were trained to conduct activities outlined in the protocol on 2/10/2020. The personnel identification was signed on a later date. att
5/20/2020



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ATTACHMENT 2 CYTOTOXICITY FINAL REPORT



Siemens Healthineers
685 East Middlefield Road,
Mountain View, CA 94043
ATTN: Reginald Rumwell

705 Front Street
Toledo, OH 43605
Phone: 419-693-5307
Fax: 419-691-0418
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Lab # 2001149
PO # 1502013412

Test Article: Protocol 19-L115: Cytotoxicity of the Konica Minolta 9VE4 4D EC Transducer Supplied
by Siemens Healthineers with Trophon 2 System
Part # 11289564 Lot # N/A Batch # N/A

ISO 10993-5 CYTOTOXICITY TEST – ELUTION METHOD FINAL REPORT

Test article received: 2/14/2020

Test start date: 2/18/2020

Test termination date: 2/21/2020

SOP No. (current version): LEXLP-047

Materials:

Test Article: A 105.18 g portion of test article was covered with 35.06 mL of Minimum Essential Medium supplemented with 5% serum, 1% antibiotics and L-glutamine (MEM) and extracted at 37°C for 24 hours.

Blank Control: A single aliquot of MEM without test article was subjected to the same conditions as described for the test article.

Negative Control: High Density Polyethylene (HDPE) was extracted in MEM using the same conditions as described for the test article.

Positive Control: The current LexaMed positive control, latex, was extracted in MEM using the same conditions as described for the test article.

Conditions of Extracts:

Test Article:	Red, clear	Negative Control:	Red, clear
Blank Control:	Red, clear	Positive Control:	Pink, cloudy

Procedure:

Triplicate 5 mL volumes of the test extract, reagent control, negative control, and positive control were added to individual flasks containing a confluent monolayer of L929 mouse fibroblast cells. The flasks were incubated at 37°C in 5% CO₂ for 48 hours. Following incubation, the cultures were examined microscopically.

The results are presented below and apply to the sample tested.



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Siemens Healthineers

705 Front Street
Toledo, OH 43605
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Lab # 2001149

Evaluation Criteria:

The confluence of the monolayer was recorded as (+) or (-). The color of the test extract was observed and compared to the negative control to evaluate if a pH shift had occurred in the test extract. Each flask was evaluated for percent lysis and cellular characteristics as follows:

Grade	Reactivity	Observations
0	None	Discrete intracytoplasmic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Results:

Flask	Confluent Monolayer	% Rounding	% Cells without Intracytoplasmic Granules	% Lysis	Grade	Reactivity
Test (1)	+	< 5	< 5	< 5	0	None
Test (2)	+	< 5	< 5	< 5	0	None
Test (3)	+	< 5	< 5	< 5	0	None

pH Observation: Neutral

The Blank Control, Negative Control, and Positive Control all performed as anticipated.

Conclusion: Under the conditions of this test, the MEM extract of the test article showed no evidence of cell lysis or toxicity. The requirements of the test, per the referenced test procedure and ISO 10993-5 guidelines, were met since the grade was ≤ 2 (Mild).

Record Storage: All raw data pertaining to this study will be maintained in the LexaMed archives for a minimum of 15 years.

Approved by J. M. Arellano Tech: EO Date 02/27/20

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**ATTACHMENT 3
EXECUTION COPY OF PROTOCOL 19-L115**



Protocol

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Originator: Erin Huber

Date: 2/3/2020

LexaMed Approvals

Study Director: Erin Huber
Printed Name Erin Huber
Signature Date: 02/03/2020

Technical Review: Anne Schuler
Printed Name Anne Schuler
Signature Date: 1-3-20

Quality Assurance: Michael Sugg
Printed Name Michael Sugg
Signature Date: 2-3-2020

Siemens Healthineers Approval(s)

See next page for approval. UM 2/7/2020

Printed Name Signature Date:
Printed Name Signature Date:

1. Purpose

- 1.1. The purpose of this study is to validate the Trophon® 2 as an automated high level disinfection system for the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers. Testing will be conducted using the following guidance documents:
 - 1.1.1. FDA guidance document: *Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling* (March 2015)
 - 1.1.2. AAMI TIR 12 – *Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for medical device manufacturers*.
 - 1.1.3. ANSI/AAMI/ISO 17664:2017 – *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*.
- 1.2. The 9VE4 4D EC Transducer will be inoculated with a *Mycobacterium terrae* microorganism suspension. The efficacy of the automated disinfection process will be assessed based on residual levels of the microorganism marker.

2. Scope

- 2.1. This study will substantiate an automated high level disinfection process, using the Trophon® 2 system for the patient contact surface of the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers.

3. Background

- 3.1. The 9VE4 4D EC Transducer is an endocavity probe produced by Konica Minolta, compatible with Siemens Healthineers ACUSON Sequoia ultrasound systems.
- 3.2. Nanosonics currently produces two (2) Trophon® systems. Both are automated, closed systems that use a sonicated hydrogen peroxide (Sonex-HL®) mist in a seven (7) minute cycle to achieve high level disinfection.
 - 3.2.1. Both Trophon® systems were previously tested by LexaMed on Siemens Healthineers semi-critical transducers producing similar results therefore only one (1) system will be used for this validation .



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Protocol

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Originator: Erin Huber

Date: 2/3/2020

LexaMed Approvals

Study Director:	Erin Huber Printed Name		Date: 02/03/2020
Technical Review:	Anne Schuler Printed Name		Date: 1-3-20
Quality Assurance:	Michael Sugg Printed Name		Date: 2-3-2020

Siemens Healthineers Approval(s)

Rumwell Printed Name		Digitally signed by Rumwell Reginald DN: CN=rumwell=Z002VKCB, givenName=Reginald, sn=Rumwell, o=Siemens, cn=Rumwell Reginald Date: 2020.02.06 13:27:08 -08'00' Signature	Date: _____
Reginald Printed Name		Signature	Date: _____

1. Purpose

- 1.1. The purpose of this study is to validate the Trophon® 2 as an automated high level disinfection system for the Konica Minolta 9VE4 4D EC Transducer supplied by Siemens Healthineers. Testing will be conducted using the following guidance documents:
 - 1.1.1. FDA guidance document: *Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling* (March 2015)
 - 1.1.2. AAMI TIR 12 – *Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for medical device manufacturers*.
 - 1.1.3. ANSI/AAMI/ISO 17664:2017 – *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*.
- 1.2. The 9VE4 4D EC Transducer will be inoculated with a *Mycobacterium terrae* microorganism suspension. The efficacy of the automated disinfection process will be assessed based on residual levels of the microorganism marker.

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- 3.2. Nanosonics currently produces two (2) Trophon® systems. Both are automated, closed systems that use a sonicated hydrogen peroxide (Sonex-HL®) mist in a seven (7) minute cycle to achieve high level disinfection.
 - 3.2.1. Both Trophon® systems were previously tested by LexaMed on Siemens Healthineers semi-critical transducers producing similar results therefore only one (1) system will be used for this validation .

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- 3.2.2. The representative system chosen by Siemens Healthineers for automated high level disinfection is the Trophon® 2 system.
- 3.2.3. The Sonex-HL® disinfection solution supplied for this validation testing was produced in September 2018 with a hydrogen peroxide concentration of 36% at filling. Per the manufacturer, the hydrogen peroxide concentration is expected to drop 1-2% over its shelf life of two (2) years, therefore, the concentration of Sonex-HL® to be used in this study will most likely be 34-35% at the time of testing.
- 3.3. This study will evaluate the efficacy of the chemical disinfection process used for component surfaces that represent the areas of the 9VE4 most likely to come into contact with the patient during routine use.
- 3.4. The efficacy of the chemical disinfection process will be evaluated based on the following features:
 - 3.4.1. Verification of the ability to effectively neutralize residual disinfectant and recover injured organisms without adversely affecting the viability of the test organisms.
 - 3.4.2. Inoculation of the test article with *Mycobacterium terrae* followed by an evaluation to determine the \log_{10} reduction of the microorganism achieved by the disinfection process.
- 3.5. Per AAMI TIR 12, validation of a HLD requires demonstration of a 6 log reduction of a *Mycobacterium* organism inoculated on to test devices. As illustrated in AAMI TIR 12, section 5.2.2, Table 2 *Microorganisms listed in descending order of resistance to chemical sterilants and disinfectants*, Mycobacteria are more resistant than viruses, fungi and vegetative organisms, therefore the results of testing with mycobacterium applies to all less resistant organisms as listed in the table. The table originated from the Center for Disease Control (CDC) and is recognized by FDA and international regulatory bodies. A copy of the table is supplied in Attachment 9.
- 3.6. *Mycobacterium terrae* is considered a clinically relevant organism as it is a slow-growing species of *Mycobacterium* that is known to cause serious skin infections and is "relatively" resistant to antibiotic therapy associated with infections. It is slightly more resistant than *M. tuberculosis* and is considered a suitable surrogate for establishing tuberculocidal activity and is commonly used for laboratory testing.
- 3.7. Prior to the initiation of this study, the Trophon® 2 system manufacturer, Nanosonics, certified all technicians who will be operating their system.
- 3.8. The results from the testing as executed in this protocol will validate the high level disinfection procedure with the Trophon® 2 system to be provided in the Transducer Disinfection Guides for Siemens Healthineers 9VE4 4D EC Transducer.

4. Responsibilities

- 4.1. LexaMed Ltd.
 - 4.1.1. Author and approve the protocol.
 - 4.1.2. Provide personnel and materials as required to coordinate and execute the protocol.
 - 4.1.3. Obtain a Trophon® 2 system and disinfectant from the manufacturer, specifically Nanosonics.
 - 4.1.4. Author, review and approve the final report.
- 4.2. Siemens Healthineers
 - 4.2.1. Review and approve the protocol.
 - 4.2.2. Provide transducers for the validation study.
 - 4.2.3. Review the final report.

5. Materials and Equipment

- 5.1. VWR® Spec-Wipe® 3 Wipers (or equivalent non-linting wipe)
- 5.2. ENZOL® Enzymatic Detergent, Advanced Sterilization Products
- 5.3. Deionized Water (DI)
- 5.4. Sterile Deionized Water (SDI)
- 5.5. Sonicator
- 5.6. Fetal Bovine Serum Albumin (FBS)
- 5.7. Suspension of *Mycobacterium terrae* (ATCC® 15755)
- 5.8. Middlebrook OADC Enrichment Broth
- 5.9. Middlebrook 7H10 agar with Tween 80, and Lecithin (M7H10 agar)
- 5.10. Peptone Recovery Buffer
- 5.11. Lethen Broth
- 5.12. Laminar Air Flow (LAF) hood
- 5.13. Standard laboratory equipment and supplies
- 5.14. Filter, pore size 0.45µm, Nalgene or equivalent

6. Sample Identification

- 6.1. 9VE4 4D EC Transducer, P/N: 11289564

Figure 1: 9VE4 4D EC Transducer

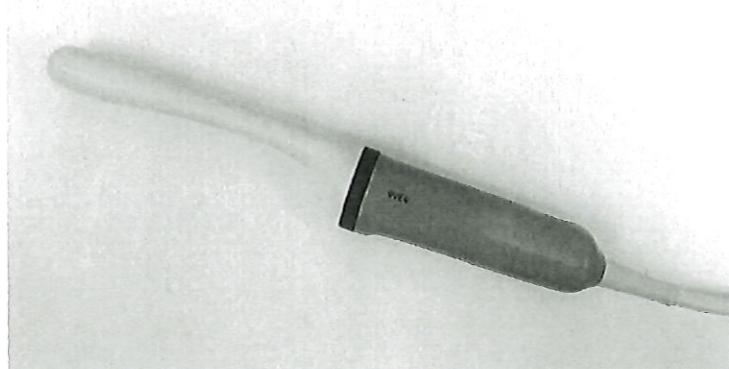
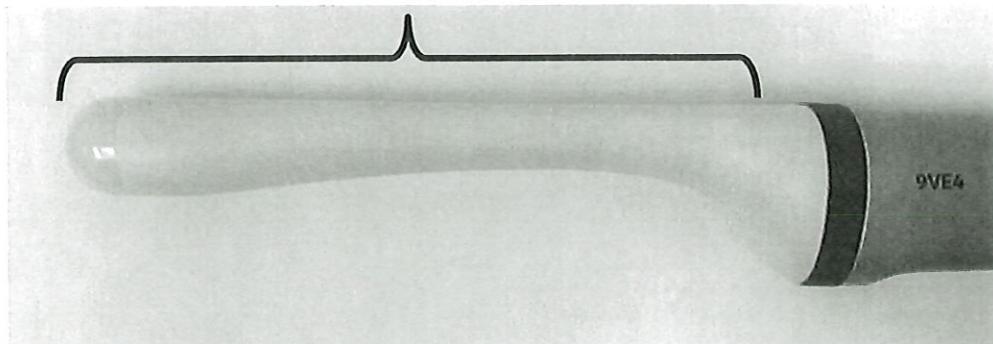


Figure 2: 9VE4 4D EC Transducer Test Area



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- 6.1.1. Only the patient contact portion of the probe as shown in Figure 2 will be tested. The handle portion will not be evaluated in this study.
- 6.2. The Sonex® HL cartridges (P/N: N05002) containing hydrogen peroxide (H_2O_2) at a concentration of 34-35% will be used within the Trophon® 2 as the automated high level disinfection system for the study. Lot number(s) used in the study will be listed in the final report.

7. Procedure

7.1. Preliminary Cleaning

- 7.1.1. The preliminary cleaning process will be conducted on each of the test samples prior to initiating the study.
NOTE: This cleaning procedure is intended ONLY for laboratory use.
- 7.1.2. Preliminary cleaning activities will be documented on Attachment 5, "Preliminary Cleaning Worksheet".
- 7.1.3. A small identification will be marked on each component to ensure traceability throughout the protocol activities.
- 7.1.4. Gloves will be worn in the detergent solution preparation and cleaning procedures.
- 7.1.5. An ENZOL® Enzymatic Detergent solution will be prepared in a container by diluting one (1) ounce (29.6 mL) of ENZOL® Enzymatic Detergent per gallon (3785 mL) of tap water at a temperature of 20-25°C. Temperatures will be verified with a calibrated thermometer.
- 7.1.6. Test components will be cleaned as a batch.
- 7.1.7. Test components will be submerged as a batch in the prepared solution for five (5) minutes.
- 7.1.8. After five (5) minutes, the test components will be removed from the detergent solution and wiped with a non-linting wipe.
- 7.1.9. Once all test components have been wiped they will be rinsed individually under running tap water at 20-25 °C for a minimum of thirty (30) seconds.
- 7.1.10. Immediately following the tap water rinse, a secondary rinse in DI water will be performed by submerging the components into a copious volume of DI water for one (1) minute. The actual volume of water utilized for the rinse will be recorded.
- 7.1.11. The test components will be wiped dry with a clean, dry wiper, and allowed to completely dry.

7.2. Validated Microbial Recovery Method

- 7.2.1. A recovery method was previously validated for this transducer under LexaMed protocol 19-L114. The validated method is outlined below:
 - 7.2.1.1. The test articles will be placed into a sterile plastic bag and covered with 500mL sterile Peptone Recovery Buffer.
 - 7.2.1.2. The sterile bags will be placed on a rotary shaker set at approximately 200 rpm for twenty (20) minutes.
 - 7.2.1.3. Based on the results from the previous method validation a recovery factor (RF) of 1.04 was established using this method. This RF will be applied to disinfection efficacy results for this study.

7.3. Verification of Disinfectant Neutralization

- 7.3.1. Prior to testing, all test articles intended for the neutralization study will be cleaned per section 7.1 then wiped with a non-linting cloth dampened with sterile isopropyl alcohol (IPA) and allowed to dry.

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7.3.2. Sufficient neutralization to allow for the enumeration of *Mycobacterium terrae*, will be verified for the Sonex HL disinfectant solution within the Trophon® 2 system. The disinfection neutralization verification procedures will be documented on Attachment 7, "Disinfectant Neutralization Verification Worksheet". Neutralization test procedures will be conducted within a LAF hood.

7.3.3. Three (3) test article replicates will be utilized for the disinfectant neutralization procedure.

7.3.4. Disinfection

7.3.4.1. Each of the three (3) test articles will be disinfected as follows:

7.3.4.1.1. Devices will be individually disinfected in the Trophon® 2 System using the manufacturer provided Sonex® HL cartridges with hydrogen peroxide at the manufacturers acceptable high end concentration of 36-37%.

7.3.4.1.2. A fresh pair of gloves will be worn by the operating technician for loading and post-disinfection removal of the device.

7.3.4.1.3. A non-linting wipe will be used to ensure the device is completely dry immediately prior to loading into the Trophon®.

7.3.4.1.4. The device will be loaded into the system's disinfection chamber, ensuring it does not come into contact with the chamber walls, and is situated above the embossed line. See Figure 3 for proper disinfection loading orientation of the 9VE4 4D EC Transducer.

Figure 3: 9VE4 4D EC Trophon® 2 Loading Orientation



7.3.4.1.5. A Trophon® chemical indicator will be placed, red side up, into the locator at the base of the chamber door.

7.3.4.1.6. The chamber door will be closed and the start button will be pressed to initiate the seven (7) minute disinfection cycle.

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- 7.3.4.1.7. At the completion of the disinfection cycle, the operating technician will verify the chemical indicator color change against the manufacturers color assessment chart on the chemical indicator carton.
- 7.3.4.1.8. The device will be removed with gloved hands, wiped with a clean, non-linting wipe, and immediately placed into an extraction vessel.
- 7.3.4.1.9. The chamber door will be closed and the device transferred to a LAF hood for testing.

7.3.5. Extraction

- 7.3.5.1. The disinfected components will be extracted using the microbial recovery method as stated in section 7.2.

7.3.6. Neutralization Efficacy

- 7.3.6.1. A working suspension of *Mycobacterium terrae* will be prepared in a 5% Fetal Bovine Serum (FBS) / Lethen Broth (LB) to target approximately 100 CFU/0.1 mL.
- 7.3.6.2. Extraction containers will be agitated to mix at the time of filtration.
- 7.3.6.3. The extraction fluid will be directly inoculated with 0.1 mL of the microbial suspensions prepared in section 7.3.6.1. The fluid from each extraction will be separately filtered through a 0.45 µm filter. Filters will be rinsed three (3) separate times with an appropriate volume of Lethen Broth (LB). Filters will then be plated as follows:
 - 7.3.6.3.1. The filters will be transferred to individual pre-poured M7H10 with T80 and lecithin agar plates that will be supplemented with Middlebrook OADC enrichment broth (approximately 1 mL Middlebrook OADC enrichment broth per 9 mL M7H10 agar). One (1) negative control will be similarly prepared and plated.

7.3.6.4. Toxicity Control Plates

- 7.3.6.4.1. Toxicity control plates will be prepared to verify that there is no negative impact of the disinfectant efficacy neutralizer solution on the population of the challenge organism.
- 7.3.6.4.2. Three (3) identical aliquots of the sterile peptone utilized for the extraction procedures will be transferred into individual sterile containers. The samples will be similarly filtered, inoculated, and plated as the extract solutions described in section 7.3.6.3 utilizing the Lethen Broth neutralizer rinses.

7.3.6.5. Viability Controls

- 7.3.6.5.1. An inoculum verification of the microbial suspension prepared in section 7.3.6.1 will be conducted in triplicate by inoculation of the suspension into a 100 mL aliquot of peptone then filtering. Filters will be plated as described in sections 7.3.6.3.1.

- 7.3.7. Plates will be incubated at 34-38°C for 7-14 days or until colonies are readily visualized.
- 7.3.8. Following incubation, all plates will be enumerated, results will be recorded, and the percent (%) recovery will be determined by comparing the Neutralizer Efficacy plate counts and the Toxicity Control plate counts with those obtained from the Viability Control plate counts.

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- 7.3.9. The following equation will be used to calculate the Neutralizer Efficacy and the Toxicity percent recoveries:

$$\frac{\text{Average Recoverable CFU (average of efficacy replicates OR average of toxicity control replicates)}}{\text{Average Recoverable CFU (average of viability control replicates)}} \times 100\%$$

- 7.3.10. The recovered test organisms from the neutralization verification should result in an average recovery of $\geq 70\%$ for the organism as compared to the average of the inoculum verification. The recovered organisms from the toxicity controls should result in an average recovery of $\geq 70\%$ for the organism as compared to the average of the inoculum verification. If the recovery does not meet the $\geq 70\%$ requirement, the neutralization system may be modified until a method recovering $\geq 70\%$ of the inoculated population is achieved.

7.4. Disinfection Efficacy Testing

- 7.4.1. Prior to disinfection efficacy testing, all test articles intended for disinfection efficacy testing will be cleaned per section 7.1 then wiped with a non-linting cloth dampened with sterile isopropyl alcohol (IPA) and allowed to dry in a LAF hood.
- 7.4.2. Disinfection efficacy testing procedures will be documented on Attachment 8, "Disinfection Efficacy Testing".
- 7.4.3. Three (3) complete disinfection efficacy determination cycles will be conducted. Each cycle will consist of microbial inoculation of the device surface, disinfection, and disinfection efficacy determination as follows. Testing will be conducted within a LAF hood.

7.4.4. Inoculation Procedure

- 7.4.4.1. A suspension of *M. terrae* will be prepared in a 5% FBS/LB solution to achieve a target total microbial population of approximately 10^7 CFU/0.1mL. A population verification of the microbial suspension will be conducted via standard dilution and plating per LEXLP-054, "Standard Plate Count for Total Bacteria and Total Fungi in Liquid and Water Soluble Products". The population verification results will be documented on Attachment 6.
- 7.4.4.2. Four (4) components will each be inoculated with a total of 0.1 mL of the microbial suspension, as prepared in section 7.4.4.1, distributed at the locations determined to be the most difficult to disinfect. The inoculation locations will be recorded on Attachment 8.
- 7.4.4.3. The inoculated surfaces will dry for a time not to exceed thirty (30) minutes.
- 7.4.4.4. One (1) test article will remain un-inoculated to serve as a negative control.

7.4.5. Disinfection Procedure

- 7.4.5.1. Each of the three (3) inoculated test articles and one (1) un-inoculated negative control test article will be disinfected as follows:
 - 7.4.5.1.1. Devices will be individually disinfected in the Trophon® 2 System using the manufacturer provided Sonex® HL cartridges with a concentration of 34-35% hydrogen peroxide.
 - 7.4.5.1.2. A fresh pair of gloves will be worn by the operating technician for loading and post-disinfection removal of the device.
 - 7.4.5.1.3. A non-linting wipe will be used to ensure the device is completely dry immediately prior to loading into the Trophon®.
 - 7.4.5.1.4. The device will be loaded into the system's disinfection chamber, ensuring it does not come into contact with the chamber walls, and is situated above the embossed line. See Figure 3 for proper disinfection loading orientation of the 9VE4 4D EC Transducer.



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- 7.4.5.1.5. A Trophon® chemical indicator will be placed, red side up, into the locator at the base of the chamber door.
 - 7.4.5.1.6. The chamber door will be closed and the start button will be pressed to initiate the seven (7) minute disinfection cycle.
 - 7.4.5.1.7. At the completion of the disinfection cycle, the operating technician will verify the chemical indicator color change against the manufacturers color assessment chart on the chemical indicator carton.
 - 7.4.5.1.8. The device will be removed with gloved hands, wiped with a clean, non-linting wipe, and immediately placed into an extraction vessel.
 - 7.4.5.1.9. The chamber door will be closed and the device transferred to a LAF hood for testing.
- 7.4.5.2. One (1) inoculated test article will not be disinfected and will be utilized as the positive control.

7.4.6. Disinfection Efficacy Determination

- 7.4.6.1. The disinfected test articles and negative control and the non-disinfected positive control component will be transferred to extraction vessels and extracted using the method as determined in section 7.2.
- 7.4.6.2. The entire extraction volume from the three (3) inoculated and disinfected test articles and the negative control will be filtered, rinsed, and directly plated.
- 7.4.6.3. The extract from the positive control will be serially diluted as required to determine the population. Duplicate appropriate volume aliquots from designated dilutions will be similarly filtered, rinsed, and plated.
- 7.4.6.4. Filters will be plated onto M7H10 with T80 and lecithin plates supplemented with Middlebrook OADC enrichment broth (approximately 1 mL Middlebrook OADC enrichment broth per 9 mL M7H10 agar). Plates will be incubated at 34-38°C for 7-14 days or until colonies are readily visualized. Following incubation, plates will be enumerated, counts recorded, and log reductions calculated on Attachment 8.
- 7.4.6.5. After recovery and prior to subsequent use, the test articles will be cleaned as defined in section 7.1.

7.5. Cytotoxicity Testing

- 7.5.1. One (1) device will be disinfected as described in section 7.4.5 then submitted for Cytotoxicity testing per LexaMed SOP LEXLP-047, "Cytotoxicity Elution Test – USP and ISO Methods" to evaluate any remaining disinfectant residuals for toxicity. Disinfection procedures will be documented on Attachment 10, "Disinfection Procedure for Cytotoxicity Testing".

7.5.2. A copy of the test report will be included in the protocol final report.

8. Acceptance Criteria

- 8.1. The disinfectant neutralization procedure method as developed in section 7.3 must successfully result in $\geq 70\%$ microbial recovery from the test article in comparison to the average inoculum verification results to support that the challenge organism can be recovered in the presence of the residual disinfectant.
- 8.2. Disinfection Efficacy Testing
 - 8.2.1. The disinfectant is being evaluated as an HLD which requires a minimum $6 \log_{10}$ reduction of mycobacterium per AAMI TIR 12. The inoculated positive control must result in a viable recovered challenge population of $\geq 10^6$ total CFU for *M. terrae* to demonstrate the capability of the disinfection process to achieve a minimum $6 \log_{10}$ reduction.

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- 8.2.2. Each disinfection process must achieve a minimum $6 \log_{10}$ reduction of *M. terrae* as compared to the positive control article in order for the disinfectant to meet the requirement of a High Level Disinfectant.
- 8.2.3. The negative controls must demonstrate < 3 CFU of *M. terrae*. Any growth recovered will be identified by colony morphology and acid-fast staining to determine if growth is indicative of *M. terrae*.
- 8.2.4. The disinfected test articles tested for cytotoxicity test must be non-cytotoxic.

9. Documentation

- 9.1. All persons conducting work associated with this protocol will be identified on Attachment 1, "Personnel Identification/Training List".
- 9.2. Deviations to the protocol procedure or acceptance criteria will be documented on Attachment 2, "Deviation Report".
- 9.3. Additional data will be documented on Attachment 3, "General Data Sheet".
- 9.4. Equipment and materials used information will be documented on Attachment 4, "Equipment and Materials Worksheet".
- 9.5. Preliminary cleaning procedures will be documented on Attachment 5, "Preliminary Cleaning Worksheet".
- 9.6. Working suspension preparation and inoculum verification procedures will be documented on Attachment 6, "Working Suspension Preparation and Inoculum Verification Worksheet".
- 9.7. Disinfectant neutralization verification procedures will be documented on Attachment 7, "Disinfectant Neutralization Verification Worksheet".
- 9.8. Disinfection efficacy determination testing procedures and results will be documented on Attachment 8, "Disinfection Efficacy Testing".
- 9.9. Disinfection procedures for cytotoxicity testing will be documented on Attachment 10, "Disinfection Procedure for Cytotoxicity Testing".

10. References

- 10.1. AAMI TIR 12 – *Designing, testing, and labeling reusable medical devices for reprocessing in health care facilities: A guide for manufacturers – 3rd edition*.
- 10.2. Guidance for Industry and Food and Drug Administration Staff: *Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling* (March 2015).
- 10.3. ANSI/AAMI/ISO 17664:2017 – *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*.
- 10.4. LexaMed SOP LEXLP-054, "Standard Plate Count for Total Bacteria and Total Fungi in Liquid and Water Soluble Products".
- 10.5. SOP LEXLP-047, "MEM Elution Cytotoxicity Test – USP and ISO Methods".
- 10.6. LexaMed protocol 19-L114, "Validation of Recovery Extraction Methods for use in Cleaning and High Level Disinfectant Validation Studies for the Konica Minolta 9VE4 4D EC Transducer Supplied by Siemens Healthineers".



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11. Attachments

- 11.1. Attachment 1: Personnel Identification / Training List
- 11.2. Attachment 2: Deviation Report
- 11.3. Attachment 3: General Data Sheet
- 11.4. Attachment 4: Equipment and Materials Worksheet
- 11.5. Attachment 5: Preliminary Cleaning Worksheet
- 11.6. Attachment 6: Working Suspension Preparation and Inoculum Verification Worksheet
- 11.7. Attachment 7: Disinfectant Neutralization Verification Worksheet
- 11.8. Attachment 8: Disinfection Efficacy Testing
- 11.9. Attachment 9: AAMI TIR 12 Table 2
- 11.10. Attachment 10: Disinfection Procedure for Cytotoxicity Testing



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ATTACHMENT 1

Each person who will be recording data on any worksheet included in this protocol must complete an entry on this page. Signatures signify they have read the protocol and understand the objectives, acceptance criteria, and procedural requirements defined prior to protocol execution.

Reviewed by: _____

Date: _____



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ATTACHMENT 2
DEVIATION REPORT
#_____

Deviation from:	() Procedure () Acceptance Criteria
Describe deviations and any immediate investigation	
Written by:	Date:
Describe recommended action, investigation and rationale	
Written by:	Date:
Study Director/Dept. Manager and Client Approval	
Approved By:	Date:
Client Approval:	Date:
Describe closure of deviation:	
Written by:	Date:
Approval of final disposition/resolution by Study Director/Dept. Manager and QA	
Approved By:	Date:
QA:	Date:



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ATTACHMENT 3

Reviewed by: _____

Date: _____



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ATTACHMENT 4 EQUIPMENT AND MATERIALS WORKSHEET

The following equipment and materials were utilized for testing on _____ (date).

Equipment/Materials:

Item	Calibration ID	Calibration Due
Thermometer		
Timer		
Pipettor(s)		
34-38°C Incubator		
Rotary Shaker		

Item	Lot/Part No.	Exp. Date
DI Water (DIW)		
VWR® Spec-Wipe® 3 Wipers		NA
ENZOL® Enzymatic Detergent		
IPA		
<i>M. terrae</i> stock suspension		NA
Sonex HL Cartridge		
Trophon® Chemical Indicator		
Fetal Bovine Serum		
Lethen Broth		
M7H10 w/ T-80 & lecithin plates (pour date: _____)*		
Middlebrook OADC Enrichment Broth		
S-DI		
Nalgene Filters		
Peptone		

*Prepared M7H10 plates were made with approximately 1mL Middlebrook OADC Enrichment Broth per 9mL M7H10 agar.

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____



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ATTACHMENT 5
PRELIMINARY CLEANING WORKSHEET

Date: _____

Test Component IDs: _____

Test component(s) was/were cleaned as follows:

Detergent Preparation:

____ mL of ENZOL® Enzymatic Detergent was added to ____ gallon(s) (_____ L) of tap water (20-25°C).
Temperature: _____ °C

Cleaning Procedure:

AS A BATCH, test components were submerged in the prepared solution for five (5) minutes.

After five (5) minutes, the components were removed from the detergent and wiped with a non-linting wipe.

Components were INDIVIDUALLY rinsed under running tap water of 20-25°C (Temperature: _____ °C) for a minimum of thirty (30) seconds.

AS A BATCH, components were submerged in _____ mL DI water for one (1) minute.

Five (5) Minute Detergent Soak		Thirty (30) Seconds Tap Water Rinse		One (1) Minute DI Water Rinse	
Start	Stop	Start:	✓ Indicates Task Completed	Start	Stop
		Device ID			
		Stop:			

Components were wiped with a clean, dry wiper and allowed to completely dry.

Conducted by: _____

Date: _____

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ATTACHMENT 6 WORKING SUSPENSION PREPARATION AND INOCULUM VERIFICATION WORKSHEET

Date: _____

The following working suspensions and IVs were conducted.

5% FBS Solution Preparation:

A 5% FBS solution was prepared by diluting _____ mL FBS into _____ mL LB

Microbial Suspension Preparation Calculations (check mark all that apply):

Where: C1 = Stock Population, C2 = Desired Population, V2 = Desired Volume, V1 = Required Volume of Stock

_____ Neutralization Microbial Suspension Preparation (100 CFU/0.1 mL):

$$C1V1 = C2V2 \Rightarrow V1 = \frac{C2V2}{C1} \Rightarrow V1 = \left(\frac{100 \text{ CFU}/0.1mL)(10 mL)}{CFU/mL} \right) = \text{_____ mL (or } \text{_____ mL from } \text{_____ dilution)} \\ \text{into } \text{_____ mL 5\% FBS/LB}$$

An IV was conducted as follows:

_____ Disinfection Efficacy Testing Working Suspension Preparation – *M. terrae* (10⁷ CFU/0.1 mL):

$$C1V1 = C2V2 \Rightarrow V1 = \frac{C2V2}{C1} \Rightarrow V1 = \left(\frac{CFU/0.1mL)(10 mL)}{CFU/mL} \right) = \text{_____ mL (or } \text{_____ mL from } \text{_____ dilution)} \\ \text{into } \text{_____ mL 5\% FBS/LB}$$

An IV was conducted as follows:

Incubation Start Time/Date/Tech: _____

Results:

_____ Neutralization Microbial Suspension Preparation IV

Incubation Stop Time/Date/Tech: _____

CFU Counts			Mean CFU	Population
				CFU/0.1 mL

_____ Disinfection Efficacy Testing Working Suspension Preparation IV – *M. terrae*:

Incubation Stop Time/Date/Tech: _____

Dilution	Volume Filtered	CFU Counts			Mean CFU	Population
10 ⁻¹						CFU/0.1 mL
10 ⁻²						

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____



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ATTACHMENT 7 DISINFECTANT NEUTRALIZATION VERIFICATION WORKSHEET

Date: _____

Disinfectant Identification: _____

Immediately prior to testing, the components were placed in a LAF hood and wiped/sprayed with IPA and allowed to dry. Tech/Date: _____

Inoculum Preparation:

An ~ 10^2 CFU / 0.1 mL microbial suspension was prepared in 5% FBS / LB (see Attachment 6).

Disinfection:

Three (3) devices (ID: _____) were individually disinfected as follows:

A clean, non-linting wipe was used to completely dry the device.

The device and chemical indicator were loaded into the disinfection chamber.

The chamber door was closed and start button pressed.

At the completion of the disinfection cycle, the chemical indicator was examined and device was removed.

The disinfected device was wiped with a clean, non-linting wipe then immediately placed in the extraction vessel and transferred to a LAF hood for testing.

Device ID	Trophon® Cycle #	Trophon® "Cycle Complete" Displayed (Yes / No)	Chemical Indicator Color Change (Pass / Fail)	Tech/Date

Recovery:

Vessel: Sterile Plastic Bag Volume Peptone: 500 mL Extraction Parameters: 200 rpm for 20 minutes

Start time: _____ Stop time: _____

Test Extract Testing Procedure:

The test extracts were inoculated with 0.1 mL of the prepared microbial suspension and filtered. Filters were rinsed three (3) times with _____ mL of the Lethen Broth.

A toxicity control of the suspension was conducted by filtration in triplicate. Three (3) _____ mL aliquots of peptone utilized for extraction procedures were transferred into individual sterile containers. The samples were similarly inoculated and tested as above.

An IV was conducted in triplicate by inoculation of the 0.1 mL inoculation volume into a 100 mL aliquot of peptone then filtering.

One (1) negative control consisting of _____ mL peptone was similarly filtered. Filters were retrieved and plated onto Middlebrook 7H10 (M7H10) with T80 and lecithin for enumeration. The plates will have been supplemented with OADC enrichment broth, approximately 1 mL OADC enrichment broth per 9 mL M7H10 agar. Plates were incubated at 34-38°C for 7-14 days or until readily visible. Incubation information is recorded on Attachment 6.

Results:

Sample ID	Recovered CFU	Average	% Recovered Avg vs. IV Avg	Acceptable Yes / No
Component #				
Component #				
Component #				
Toxicity #1				
Toxicity #2				
Toxicity #3				
IV (See Attachment 6)			Negative control: _____	

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____



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**ATTACHMENT 8
DISINFECTION EFFICACY TESTING**
Page 1 of 2

Date: _____

Cycle No. _____

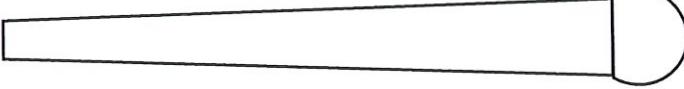
Immediately prior to testing, the components were placed in a LAF hood and wiped/sprayed with IPA and allowed to dry. Tech/Date: _____

Inoculum Preparation and Inoculation Procedure:

A working suspension at $\sim 10^7$ CFU / 0.1 mL *M. terrae* was prepared in 5% FBS/LB (see Attachment 6).

Each of four (4) components (_____, ____, ____, ____) were inoculated with _____ mL of the prepared suspension at each of _____ locations on the component for a total inoculum volume of _____ mL and allowed to dry for not greater than 30 minutes.

Inoculation Locations:

(Side View) 

(Head View) 

Dry Start: _____ Dry Stop: _____

Disinfection:

Three (3) devices (ID: _____) and one (1) negative control (ID: _____) were individually disinfected as follows:

A clean, non-linting wipe was used to completely dry the device.

The device and chemical indicator were loaded into the disinfection chamber.

The chamber door was closed and start button pressed.

At the completion of the disinfection cycle, the chemical indicator was examined and device was removed.

The disinfected device was wiped with a clean, non-linting wipe then immediately placed in the extraction vessel and transferred to a LAF hood for testing.

Device ID	Trophon® Cycle #	Trophon® "Cycle Complete" Displayed (Yes / No)	Chemical Indicator Color Change (Pass / Fail)	Tech/Date

Extraction:

The test samples and negative and positive control were extracted using:

Vessel: Sterile Plastic Bag Volume Peptone: 500 mL Extraction Parameters: 200 rpm for 20 minutes

Start time: _____ Stop time: _____

Microbial Testing:

Extracts from the three (3) inoculated and disinfected components and the negative control were filtered. Filters were rinsed _____ times with _____ mL aliquots of _____, then filters were placed on pre-poured plates.

Positive Control Processing-

Duplicate aliquots of the extract were filtered. Dilutions, if required, were prepared in S-DI. Testing was conducted as follows: M7H10 plates were incubated at 34-38°C for 7-14 days or until readily visible. See Attachment 6 for incubation information.

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____



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ATTACHMENT 8 DISINFECTION EFFICACY TESTING Page 2 of 2

Date: _____

Cycle No. _____

Results:

Positive Control (PC) Component Testing:

Dilution	Volume Filtered	Recovered CFU	Recovered Population	Extraction Volume	Total Population ¹	Log of PC Population
10 ⁻						
10 ⁻				500 mL		

$$^1 \text{Population} = \left(\frac{\text{Recovered Population CFU}}{\text{Volume Filtered}} \right) \times \left(500 \text{ mL (Extraction Volume)} \right)$$

Disinfection Efficacy Test Components:

Sample ID	Actual Recovered CFU	Volume Filtered (mL)	Corrected Population CFU ²	Log of Recovered CFU	Log of Corrected PC Population	Log Reduction Achieved ³	Log Reduction Required	Requirement Met (Yes / No)
								<input type="checkbox"/> Yes <input type="checkbox"/> No
								<input type="checkbox"/> Yes <input type="checkbox"/> No
								<input type="checkbox"/> Yes <input type="checkbox"/> No
Neg. Control								<input type="checkbox"/> Yes <input type="checkbox"/> No

$$^2 \text{Corrected Population CFU} = \left(\frac{\text{Total Recovered CFU}}{\# \text{ of Replicates}} \right) \times \left(\frac{500 \text{ mL (Total Extract Volume)}}{\text{Volume Filtered}} \right)$$

$$^3 \text{Log Reduction Calculation} = \text{Log of Positive Control Component} - \text{Log of Corrected Population}$$

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____



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ATTACHMENT 9
AAMI TIR 12, Table 2

Table 2—Microorganisms listed in descending order of resistance to chemical sterilants and disinfectants¹⁾

Bacterial spores <i>Geobacillus stearothermophilus</i> ²⁾ <i>Bacillus subtilis</i> <i>Bacillus atrophaeus</i> ³⁾ <i>Clostridium sporogenes</i>
Cyst forms of parasites <i>Cryptosporidium</i> cysts
Mycobacteria <i>Mycobacterium tuberculosis</i> var. <i>bovis</i> Nontuberculous mycobacteria ⁴⁾
Nonlipid or small viruses Poliovirus Coxsackie virus Rhinovirus
Fungi <i>Trichophyton</i> spp. <i>Cryptococcus</i> spp. <i>Candida</i> spp.
Non-cyst forms of parasites
Vegetative bacteria <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i> <i>Salmonella choleraesuis</i> Enterococci
Lipid or medium-sized viruses Herpes simplex virus Cytomegalovirus Respiratory syncytial virus Hepatitis B virus Hepatitis C virus Human immunodeficiency virus

NOTE 1—The order of resistance is given as a guide and can vary depending on the microorganism and the chemical sterilant or disinfectant.

NOTE 2—Formerly *Bacillus stearothermophilus* (see Nazina, et al., 2001).

NOTE 3—Strains now designated *Bacillus atrophaeus* were formerly classified as *Bacillus subtilis* (see Fritze and Pukall, 2001).

NOTE 4—Some strains of nontuberculous mycobacteria have shown unique resistance to glutaraldehyde and OPA (Duarte, et al., 2009; Griffiths, et al., 1997; Svetlikova, et al., 2009).

NOTE 5—Prions, the causative agents of transmissible spongiform encephalopathies, present a unique resistance challenge to germicidal chemicals. Prions have been shown to have unusually high resistance to heat and chemicals, in some cases demonstrating greater resistance than bacterial spores. Special consideration should be given to devices contaminated in suspected or confirmed cases. For information regarding the processing of devices exposed to prions, see Annex C.



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ATTACHMENT 10 Disinfection Procedure for Cytotoxicity Testing

Disinfectant Identification: _____

Disinfection:

The test article was disinfected as outlined below:

A clean, non-linting wipe was used to completely dry the device.

The device and chemical indicator were loaded into the disinfection chamber.

The chamber door was closed and start button pressed.

At the completion of the disinfection cycle, the chemical indicator was examined and device was removed.

The disinfected device was wiped with a clean, non-linting wipe then immediately placed in the extraction vessel and submitted for cytotoxicity testing.

Device ID	Trophon® Cycle #	Trophon® "Cycle Complete" Displayed (Yes / No)	Chemical Indicator Color Change (Pass / Fail)	Tech/Date

Conducted by: _____

Date: _____

Reviewed by: _____

Date: _____