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FINAL REPORT

CLEANING EVALUATION VALIDATION

PROCEDURE NO. STP0083 REV 04
PROTOCOL DETAIL SHEET NO. 200900083 REV 01

LABORATORY NO. 462879

PREPARED FOR:

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CLEANING EVALUATION VALIDATION

LABORATORY NUMBER:	462879
PROCEDURE NUMBER:	STP0083 REV 04
PROTOCOL DETAIL SHEET NUMBER:	200900083 REV 01
SAMPLE SOURCE:	Ruhof Healthcare
SAMPLE IDENTIFICATION:	(Endozime XP Batch Number 7186 Manufactured January 22, 2009 (1 bottle)) Sponsor Supplied, 1 box Proformance (TOSI) automated washer test coupons, Sponsor Supplied P.O. #Ruhof101
DEVIATIONS:	None
NUMBER OF TEST SAMPLES:	30 (Nelson Laboratories, Inc. provided 4 additional coupons)
PROTOCOL APPROVAL DATE:	11 Feb 2009
SAMPLE RECEIVED DATE:	17 Feb 2009
LAB PHASE START DATE:	24 Feb 2009
LAB PHASE COMPLETION DATE:	02 Mar 2009
REPORT ISSUE DATE:	03 Mar 2009

INTRODUCTION:

A cleaning validation was performed using the manufacturer's recommended cleaning procedures and specifications. One positive coupon and three test coupons were utilized to determine the reduction in bioburden. An extraction was performed on the positive coupon. Three sponsor provided TOSI coupons were also cleaned and visually inspected.

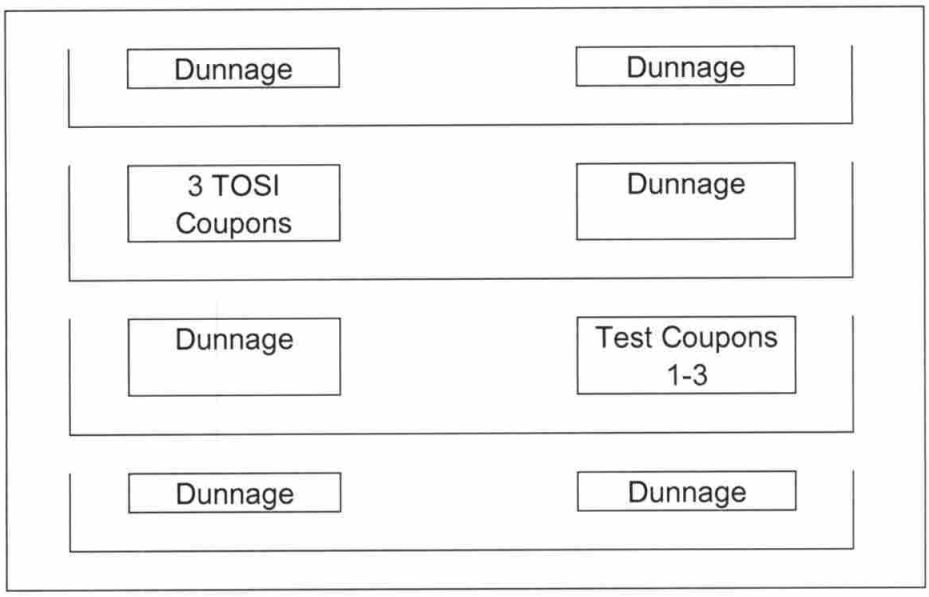
PROCEDURES:

Culture Preparation: The defibrinated blood soil (DBLSO) was inoculated with *Geobacillus stearothermophilus*, ATCC #7953 from a stock spore suspension maintained at 2-8°C to yield a minimum population of 10^4 colony forming units (CFU)/mL. A standard plate count was performed on the inoculated test soil to determine the initial titer of the test organism.

Sample Contamination: The coupons were immersed in inoculated soil and allowed to remain in contact with the soil for a minimum of 15 minutes. The soiled coupons were placed into a clean pan and the pan was covered with a towel dampened with sterile purified water (PURW). The coupons were allowed to set for a minimum of 30 minutes to simulate the wait time between contamination and cleaning.

Water Hardness Test: The water hardness was measured the day of automated procedure testing. A sample of cold water from the water inlet and a sample of hot water from the water inlet were collected in separate sterile containers. The water hardness was measured using a Model 5B Hardness Test Kit.

Cleaning Procedure: The coupons were transferred into mesh baskets and loaded onto the 4-Level manifold rack accessory contained inside the STERIS® 444 washer for processing. Sample placement as well as the dunnage placement used to simulate a maximum load is shown in the following figure:



Dunnage = ~20 scissors

The cycle was selected and the following set of parameters was properly programmed:

PHASE	RECIRCULATION TIME (MINUTES)	WATER TEMPERATURE	DETERGENT TYPE AND CONCENTRATION
Pre-Wash 1	03:00	Cold tap water	N/A
Enzyme Wash	03:00	Hot tap water	Detergent provided by the sponsor (1/4 oz/gal)
Wash 1	03:00	65.5°C (Set Point)	Detergent provided by the sponsor (1/4 oz/gal)
Rinse 1	03:00	Hot tap water	N/A
Thermal Disinfection	05:00	90°C	N/A

Upon completion of the draining phase after Rinse 1, the cycle was stopped and the coupons were removed from the washer.

The coupons were visually examined by the naked eye under normal lighting conditions to determine if all adherent visible soil (e.g. blood, protein substances and other debris) had been removed from the surfaces, lumens, crevices, and serrations. Refer to Figure 1.

Bioburden Testing: The positive and cleaned coupons were tested for bioburden by immersing in peptone Tween® (PEPT) and shaking manually 100 times to extract the organisms present. Aliquots of the extract fluid were diluted where appropriate, and plated onto soybean casein digest agar (SCDA) or filtered through a 0.45 µm membrane and the membrane was placed onto SCDA. Plates were incubated at 55-60°C for 24 ± 3 hours and colonies were enumerated.

Calculations for the Bioburden: The percent reduction in bacterial counts was calculated using the following formula:

$$\% \text{ Reduction} = 100 - \left(\frac{\text{final population}}{\text{initial population}} \times 100\right)$$

Log reductions were calculated using this formula:

$$\text{Log Reduction} = \text{Log } \textit{Initial} \text{ Population} - \text{Log } \textit{Final} \text{ Population}$$

Where: Final population = Number of organisms recovered from the cleaned coupons
Initial population = Positive coupon titer

Estimations were reported for counts which were outside of the statistically accurate range.

RESULTS:

The results of the cleaning evaluation are summarized in Table 1. Each coupon was individually inoculated, and some variability is expected between coupons. The soil titer and the positive coupon titer are listed at the bottom of the table. The TOSI coupons were compared to the TOSI chart, and all coupons were a 0 according to the TOSI chart listed in Figure 1.

Water Hardness Testing Results: Cold Water was 222.3 ppm
Hot Water was 188.1 ppm

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

John M. Rauch
Technical Reviewer

Nick Workman
Study Director

06 mar 2009
Study Completion Date

emm

0-5 T.O.S.I. chart scale

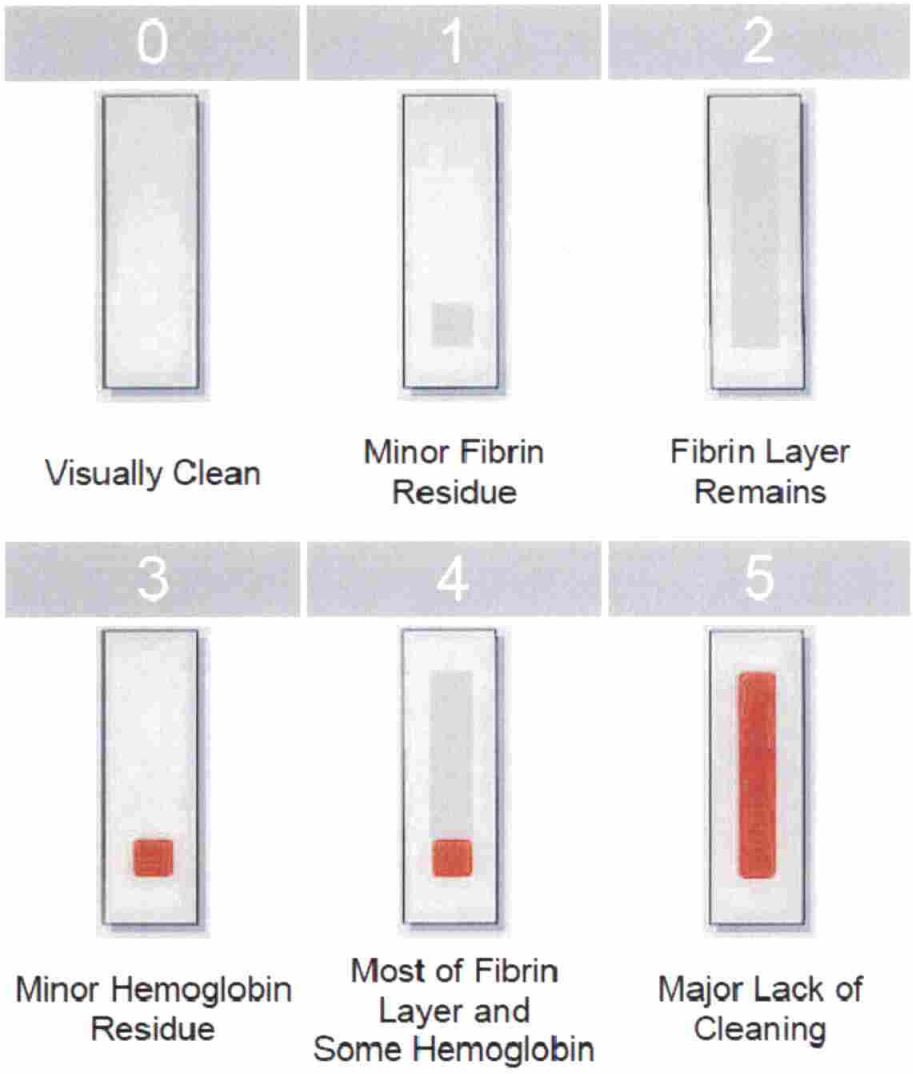


Figure 1. T.O.S.I. Chart

TABLE 1. Cleaning Results

DEVICE NUMBER	COUNTS PER DEVICE (CFU)	PERCENT REDUCTION (%)	LOG_{10} REDUCTION
1	1.1×10^0	99.99979	5.7
2	$<1.1 \times 10^0$	>99.99979	>5.7
3	$<1.1 \times 10^0$	>99.99979	>5.7

Soil Titer: 1.0×10^4 CFU/mL

Positive Device Titer: 5.2×10^5 CFU/device