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TEST RESULT CERTIFICATE

Sponsor	ndd Medical Technologies	Technical Initiation	12/15/99
Address	17 Progress Drive Chelmsford, MA 01824	Technical Completion	12/20/99
Contact	Joy Brunton	Report Date	12/27/99
P.O. Number	120899-2	Project Number	99-3741-N1

Test Article	Spirette
Lot #	Not Supplied
Study	Bacterial Challenge - Sponsor Specified

REFERENCE: ANSI/AAMI/ISO 11737-1, 1995. Sterilization of Medical Devices - Microbiological Methods -Estimation of Population of Microorganisms on Products.

United States Pharmacopoeia 24, National Formulary 19 <71> Sterility Tests pp

GENERAL PROCEDURE: *Bacillus subtilis* was streaked on Trypticase soy agar (TSA) and incubated at 30-35 °C for 18-24 hours. Isolated colonies were resuspended in sterile 0.9 % Sodium Chloride for injection (NaCl) and adjusted to approximately 1 x 10^8 Colony Forming Units (CFU) per mL using a #0.5 McFarland standard. The inoculum was diluted to 1 x 10^6 CFU/mL in sterile NaCl. This was used as the challenge solution. The concentration of challenge solution was verified using serial dilutions and standard plate counts. All plates were incubated at 30-35 °C for 18-24 hours and the number of CFU per plate were enumerated. The test article (1 unit) was inserted into the spirette holder 1 mL of the challenge suspension was applied to the inside of the spirette using a cotton swab. All surfaces were contaminated including the filter. A Pulmoguard syringe filter was attached to a three-liter syringe (to prevent contamination of the syringe). The spirette was connected to the syringe filter and pumped six times (three expiratory and three inspiratory strokes). The spirette was allowed to remain in place for 5 minutes then removed. A new syringe filter was attached to the syringe and a new spirette was inserted into the spirette holder. The spirette was attached to the syringe filter and the syringe was pumped six times (three expiratory and three inspiratory strokes). The spirette was allowed to remain in place for 5 minutes, then removed and transferred to a sterile jar. A bioburden was performed on the inside of the spirette. Each inoculated test article was rinsed with 25 mL of sterile Fluid D and shaken. The rinsate (1 mL) was individually spread plated onto separate Trypticase Soy Agar (TSA) plates and incubated at 30-35°C for 5 days. Colony Forming Units (cfu) were enumerated. This was repeated with total of ten test articles (with the challenge only done initially).
Three positive controls were tested by challenging the inside surface of the test article and performing a bioburden as described above. Negative controls (3 units) were test articles to which no challenge was done and were tested for biobuden as described above.

RESULTS: The results are summarized in Tables 1-3.

Table 1: Number of Colony Forming Units (CFU) per Test Article

Test article	1	2	3	4	5	6	7	8	9	10	Average
TSA (CFU/plate)	NOC	NOC	NOC	NOC	NOC	NOC	NOC	NOC	NOC	NOC	NOC

NOC = No observed colonies

Table 2: Number of Colony Forming Units (CFU) per Positive Control Article

Test article	Positive 1	Positive 2	Positive 3	Average
CFU/Plate	72	72	74	73
Dilution Factor	2.5×10^4	2.5×10^4	2.5×10^4	2.5×10^4
CFU/mL	1.8×10^6	1.8×10^6	1.9×10^6	1.8×10^6

Table 3: Number of Colony Forming Units (CFU) per Negative Control Article

Test article	Negative 1	Negative 2	Negative 3	Average
CFU/Plate	NOC	NOC	NOC	NOC

The average percent recovery for the positive control was calculated as

$$\frac{\text{Population Recovered}}{\text{Population Inoculated}} \times 100\% = \% \text{ Recovery } \frac{1.8 \times 10^6}{3.5 \times 10^6} \times 100 = 51.4 \% \text{ Recovery}$$

CONCLUSION: The average CFU/Test article was NOC. The average number of CFU/control was NOC and 1.8×10^6 for the negative and positive control articles respectively. The percent recovery for the positive control was 52%. The test article as tested effectively prevents cross contamination of bacteria from one contaminated spirette to the next.

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