

# BIOBURDEN AND THEORETICAL STERILITY DOSE CALCULATION FOR RADIATION STERILIZATION OF SURGICAL COTTON AND BANDAGES

Pages with reference to book, From 8 To 10

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## ABSTRACT

The initial bioburden, count of colony forming unit (CFU) was determined on the locally manufactured non-sterilized surgical cotton and bandages. In all 489 tests were conducted on 163 cotton samples and 246 tests on 82 bandage samples. The surgical cotton showed an average of 198 microbes with a maxima of 287 and minima 94 whereas bandages showed an average of 179 microbes with a maxima of 268 and minima of 89. In the 20% samples subjected to identification no anaerobic microorganism was isolated while the aerobic microorganisms isolated were all bacilli. The sterilization dose (SD) for sterility assurance level (SAL) of 10<sup>-6</sup> was 2.23 Mrads and 2.21 Mrads, whereas the device verification dose (DVD) was 0.6 and 0.59 Mrads for cotton and bandages respectively as calculated by the method proposed by the Sterilization Standard Committee, Association of Advancement of Medical Instrumentation (AAMI) (JPMA 43: 8, 1993).

## INTRODUCTION

During the last two decades manufacturers of medical products especially of single-use products are shifting from heat (autoclaving) and ethylene trioxide (ETO) gas sterilization methods to the much superior radiation sterilization methodology. This is the best technique to obtain products free of microorganisms and is now very well organized in many developed countries<sup>1,2</sup>. This technique of bulk sterilization of pre packed products is efficient, economical with only a single parameter of time/dose to control<sup>1,2</sup>. The degree of sterility achieved in autoclaving and ETO sterilization is established by testing the end product after the sterilization process. However, this sterility testing is not feasible in case of radiation sterilization of products, as it can give a false positive result<sup>3</sup>. Alternatively microbiologists have suggested the determination of initial bioburden on the item prior to sterilization, to determine the exact radiation dose needed for sterilization. Therefore it is necessary to follow good manufacturing practices (GMP) to limit the initial microbial contamination of the products so as to minimize the initial bioburden. Depending on the initial bioburden, the choice of radiation dose to sterilize disposable medical products would vary from batch to batch. In order to avoid undue exposure of items to irradiation and to make irradiation process economical and efficient, it is essential to make the proper choice of radiation dose<sup>4</sup>. A number of radiation dose calculation studies have been carried out on various materials using different approaches<sup>5-13</sup>. This work will give a picture of the microbial count present on non-sterilized disposable local products such as surgical cotton and bandages. This microbial count is used to calculate the dose required to sterilize these items to achieve a sterility assurance level (SAL) of 10<sup>-6</sup> i.e., one contaminated item out of a million, as suggested for these products by the Association of Advancement of Medical Instruments (AAMI)<sup>14</sup>. The radiation dose calculation is done by formulation proposed by AAMI and it entails prior determination of bioburden on different medical devices and radiating 100 such devices to achieve an SAL level of 10<sup>-3</sup> to 10<sup>-6</sup> so as to estimate the exact dose from the particular item depending upon its use. Thus the parameters for

determining the sterilizing dose are the average bioburden on the device and the required Sterility Assurance Level for that product. The doses are given in tabular form for different SAL levels<sup>14</sup>. If the average bioburden per device and the device SIP is not within 20% of the table given, they must be calculated by AAMI interpolation method<sup>14</sup>.

## MATERIALS AND METHODS

The chemical polysorbate, tryptone, tryptic soya agar were obtained from Difco while millipore filters 0.45 micron (gamma sterilized) and filtration system were acquired from Sartorius. The other equipment used involved laminar flow unit (bioflow model), vacuum pump, incubator, colony counter, vortex mixer, anaerobic jar, petri dishes, conical flasks and pipettes of 2 ml, 5 ml and 10 ml. All glass wares and solutions used were pre-sterilized. The samples of cotton and bandages were collected from the market in packed condition. Experiments were performed under the laminar flow cabinet according to AAMI laid down procedure and methodology<sup>15,16</sup>. 1 gram of cotton or bandage from each sample in triplicate were transferred into 250 ml conical flask containing 100 ml soaking solution (0.1% sterile saline polysorbate) and kept for 1 hour and shaken after every 15 minutes<sup>15,16</sup>. The samples were then removed and discarded after squeezing the solution off by a sterile forcep. The 2 ml, 5 ml and 20 ml aliquots of each solution were then filtered through 0.45 micron millipore filters and aliquots were raised upto 50 ml using rinsing solution (sterile 0.5% tryptone with 0.1% polysorbate). The filter discs were placed on tryptic soya agar (TSA) plates prepared one day prior to the experiment. These were incubated for 7 days at 33-35°C and one of the sets was also incubated in the aerobic jar at 33-35°C for 5 days. The total colony counts on the filter discs were multiplied by the appropriate dilution factor to obtain the estimate of the total number of viable microorganisms of CFU present in per gram of the sample. The average value of CFU calculated on cotton and bandages were 198 and 179 respectively. The value obtained was used for calculation of device verification dose (DVD) and sterilization dose (SD). Distribution of contaminants found per sample on cotton and bandages were plotted against the % frequency of microorganisms present in the samples (Figures 1 and 2). Since it was laborious to identify all the microorganisms recovered, only 20% of the samples were subjected to identification (Table I and II). Optimum radiation dose was calculated by the method<sup>4</sup> suggested by Sterilization Standard Committee, Association of the Advancement of Medical Instrumentation (AAMI), using average number of microbes found as standard in both cases<sup>14</sup>.

### Dose calculation

#### (A) Surgical cotton

- (1) Since average bioburden of 198 on surgical cotton was not within 20% value in Table B1.A and B1.B, therefore, section B 1. B of AAMI was followed<sup>4</sup>.
- (2) A verification dose SAL was determined by interpolation between 100 to 500 CFU.
- (3) Determination of Device Verification Dose (DVD) for standard item proportion (SIP) 0.1:

#### **Bioburden on device 100 198**

SIP dose (Mrads)      0.52      DVD

(i) Bioburden Extrapolation Factor (BEF)

$$= \log(198) - \log(100) \div \log(500) - \log(100)$$

(ii) Device Verification Dose (DVD)

$$= 0.42(0.71 - 0.52) + 0.52 = 0.6 \text{ Mrads}$$

(4) Sterilization Dose (SD) for SAL of 10<sup>-6</sup>

$$= 0.42(2.37 + 2.12) + 2.12 = 2.23 \text{ Mrads}$$

#### **(B) Bandages**

- (1) Since average bioburden of 179 CFU on bandages was not within 20% value in Table B1.A and B1.B, so we have followed section B1.3 of AAMI<sup>14</sup>.

- (2) A verification dose was determined by interpolation between 100 to 500 CFU.
- (3) Determination of verification dose for standard item proportion (SIP) 0.1 was done as previously.
  - (i) Bioburden extrapolation factor (BEF) = 0.36
  - (ii) Device verification dose (DVD) = 0.59 Mrads
- (4) Sterilization dose (SD) for SAL of  $10^{-5}$  = 2.21 Mrads

## RESULTS AND DISCUSSION

The maximum and minimum contaminants recorded on cotton were 287 and 94 respectively, with an average of 198 per sample. For bandages, the maximum and minimum contaminants were 268 and 89 respectively, with an average of 179 per sample. The statistical treatment of the data of contaminants found on cotton and bandages showed that the arithmetic means (198, 179), geometric means (189, 174) and medians (192, 177) respectively were similar, indicating a Gaussian distribution of contaminant on both products. This was also evident from the contaminants' frequency distribution plotted as histograms (Figure 1 and 2).

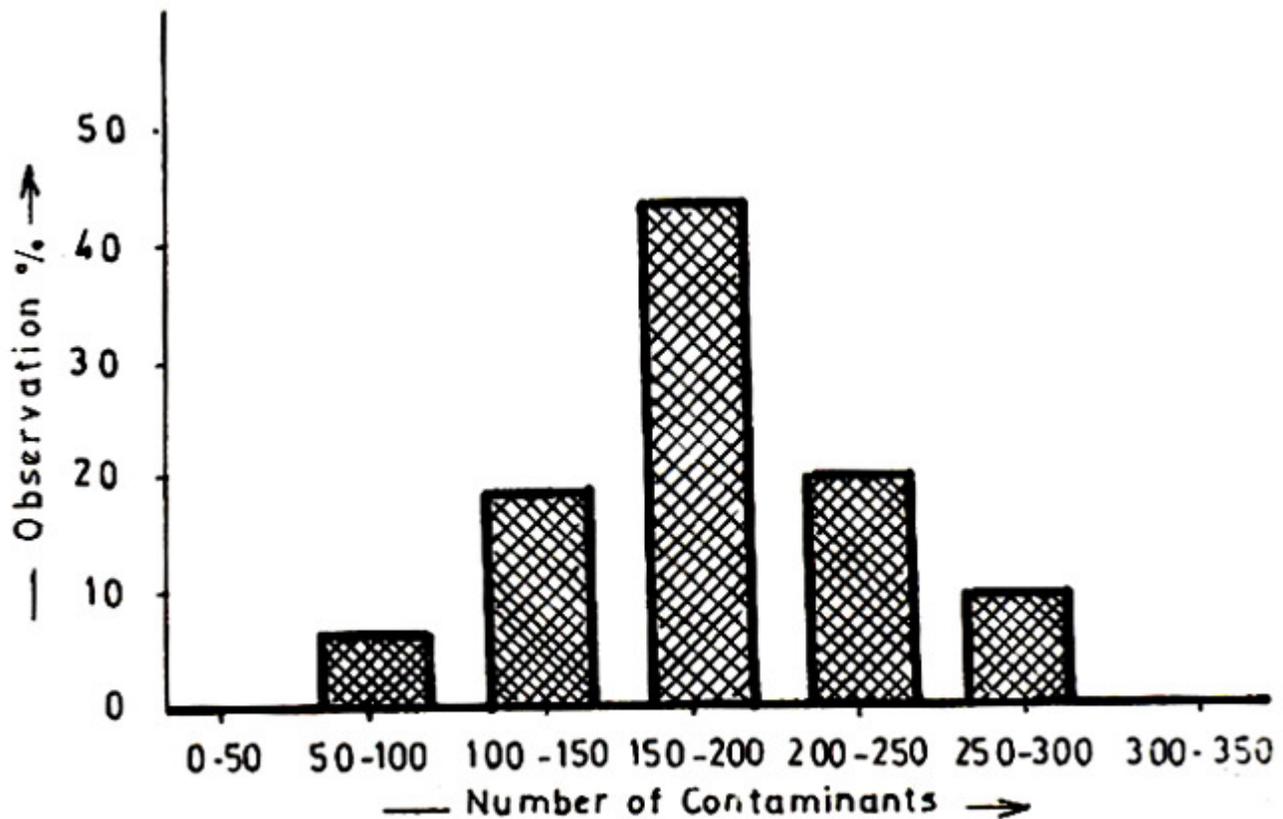


Figure 1. Frequency distribution of contaminants on surgical cotton.



The comparison of number of contaminants found on cotton as well as on bandages showed random distribution of contaminants, which was indicative of bad manufacturing practices prevailing at manufacturing sites. There was no significant difference in an average contaminants per lot. The 20% of the isolates were subjected to taxonomic studies. Most of the isolates were bacilli, of which bacillus pumilus and bacillus subtilis were predominant (Table I and II).

Order	Family	Genus	Species	No. of Isolates
1	Bacillales	Bacillus	B. pumilus	10
2	Bacillales	Bacillus	B. subtilis	8
3	Bacillales	Bacillus	B. cereus	5
4	Bacillales	Bacillus	B. pasteurii	3
5	Bacillales	Bacillus	B. thuringiensis	2
6	Bacillales	Bacillus	B. anthracis	1
7	Bacillales	Bacillus	B. clausii	1
8	Bacillales	Bacillus	B. firmus	1
9	Bacillales	Bacillus	B. licheniformis	1
10	Bacillales	Bacillus	B. pasteurii	1
11	Bacillales	Bacillus	B. thuringiensis	1
12	Bacillales	Bacillus	B. anthracis	1
13	Bacillales	Bacillus	B. clausii	1
14	Bacillales	Bacillus	B. firmus	1
15	Bacillales	Bacillus	B. licheniformis	1
16	Bacillales	Bacillus	B. pasteurii	1
17	Bacillales	Bacillus	B. thuringiensis	1
18	Bacillales	Bacillus	B. anthracis	1
19	Bacillales	Bacillus	B. clausii	1
20	Bacillales	Bacillus	B. firmus	1
21	Bacillales	Bacillus	B. licheniformis	1
22	Bacillales	Bacillus	B. pasteurii	1
23	Bacillales	Bacillus	B. thuringiensis	1
24	Bacillales	Bacillus	B. anthracis	1
25	Bacillales	Bacillus	B. clausii	1
26	Bacillales	Bacillus	B. firmus	1
27	Bacillales	Bacillus	B. licheniformis	1
28	Bacillales	Bacillus	B. pasteurii	1
29	Bacillales	Bacillus	B. thuringiensis	1
30	Bacillales	Bacillus	B. anthracis	1
31	Bacillales	Bacillus	B. clausii	1
32	Bacillales	Bacillus	B. firmus	1
33	Bacillales	Bacillus	B. licheniformis	1
34	Bacillales	Bacillus	B. pasteurii	1
35	Bacillales	Bacillus	B. thuringiensis	1
36	Bacillales	Bacillus	B. anthracis	1
37	Bacillales	Bacillus	B. clausii	1
38	Bacillales	Bacillus	B. firmus	1
39	Bacillales	Bacillus	B. licheniformis	1
40	Bacillales	Bacillus	B. pasteurii	1
41	Bacillales	Bacillus	B. thuringiensis	1
42	Bacillales	Bacillus	B. anthracis	1
43	Bacillales	Bacillus	B. clausii	1
44	Bacillales	Bacillus	B. firmus	1
45	Bacillales	Bacillus	B. licheniformis	1
46	Bacillales	Bacillus	B. pasteurii	1
47	Bacillales	Bacillus	B. thuringiensis	1
48	Bacillales	Bacillus	B. anthracis	1
49	Bacillales	Bacillus	B. clausii	1
50	Bacillales	Bacillus	B. firmus	1
51	Bacillales	Bacillus	B. licheniformis	1
52	Bacillales	Bacillus	B. pasteurii	1
53	Bacillales	Bacillus	B. thuringiensis	1
54	Bacillales	Bacillus	B. anthracis	1
55	Bacillales	Bacillus	B. clausii	1
56	Bacillales	Bacillus	B. firmus	1
57	Bacillales	Bacillus	B. licheniformis	1
58	Bacillales	Bacillus	B. pasteurii	1
59	Bacillales	Bacillus	B. thuringiensis	1
60	Bacillales	Bacillus	B. anthracis	1
61	Bacillales	Bacillus	B. clausii	1
62	Bacillales	Bacillus	B. firmus	1
63	Bacillales	Bacillus	B. licheniformis	1
64	Bacillales	Bacillus	B. pasteurii	1
65	Bacillales	Bacillus	B. thuringiensis	1
66	Bacillales	Bacillus	B. anthracis	1
67	Bacillales	Bacillus	B. clausii	1
68	Bacillales	Bacillus	B. firmus	1
69	Bacillales	Bacillus	B. licheniformis	1
70	Bacillales	Bacillus	B. pasteurii	1
71	Bacillales	Bacillus	B. thuringiensis	1
72	Bacillales	Bacillus	B. anthracis	1
73	Bacillales	Bacillus	B. clausii	1
74	Bacillales	Bacillus	B. firmus	1
75	Bacillales	Bacillus	B. licheniformis	1
76	Bacillales	Bacillus	B. pasteurii	1
77	Bacillales	Bacillus	B. thuringiensis	1
78	Bacillales	Bacillus	B. anthracis	1
79	Bacillales	Bacillus	B. clausii	1
80	Bacillales	Bacillus	B. firmus	1
81	Bacillales	Bacillus	B. licheniformis	1
82	Bacillales	Bacillus	B. pasteurii	1
83	Bacillales	Bacillus	B. thuringiensis	1
84	Bacillales	Bacillus	B. anthracis	1
85	Bacillales	Bacillus	B. clausii	1
86	Bacillales	Bacillus	B. firmus	1
87	Bacillales	Bacillus	B. licheniformis	1
88	Bacillales	Bacillus	B. pasteurii	1
89	Bacillales	Bacillus	B. thuringiensis	1
90	Bacillales	Bacillus	B. anthracis	1
91	Bacillales	Bacillus	B. clausii	1
92	Bacillales	Bacillus	B. firmus	1
93	Bacillales	Bacillus	B. licheniformis	1
94	Bacillales	Bacillus	B. pasteurii	1
95	Bacillales	Bacillus	B. thuringiensis	1
96	Bacillales	Bacillus	B. anthracis	1
97	Bacillales	Bacillus	B. clausii	1
98	Bacillales	Bacillus	B. firmus	1
99	Bacillales	Bacillus	B. licheniformis	1
100	Bacillales	Bacillus	B. pasteurii	1

TABLE I: Distribution of bioburden of 100 samples of 100 bandages

No.	Type	Bioburden	%
1	Bandage	10	10%
2	Bandage	100	10%
3	Bandage	1000	10%
4	Bandage	10000	10%
5	Bandage	100000	10%
6	Bandage	1000000	10%
7	Bandage	10000000	10%
8	Bandage	100000000	10%
9	Bandage	1000000000	10%
10	Bandage	10000000000	10%
11	Bandage	100000000000	10%
12	Bandage	1000000000000	10%
13	Bandage	10000000000000	10%
14	Bandage	100000000000000	10%
15	Bandage	1000000000000000	10%
16	Bandage	10000000000000000	10%
17	Bandage	100000000000000000	10%
18	Bandage	1000000000000000000	10%
19	Bandage	10000000000000000000	10%
20	Bandage	100000000000000000000	10%

The radiation dose calculated for SAL of  $10^{-6}$  depending on average contaminants, for cotton was 2.23 Mrads and 2.21 Mrads for bandages, whereas DVD was 0.6 Mrads and 0.59 Mrads respectively. The sterility test of 100 samples of each surgical cotton and bandages carried out after treating them at their respective DVD of 0.6 and 0.59 gave no positive results, thus verifying the correctness of the calculated SAL dose of 2.23 Mrads and 2.21 Mrads respectively for cotton and bandages. The average number of contaminants found per sample clearly indicates that the manufacturers are not following good manufacturing practices (GMP) codes as compared to the developed countries where the reported<sup>7,10-12</sup> average bioburden is much lower. In order to upgrade the standard of health care, this industry should subject their products to sterilization, especially to radiation sterilization and cotton, gauze and bandages should be supplied in small single use packing to avoid contamination when once opened.

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