THE PRESTERILIZATION MICROBIAL LOAD ON USED MEDICAL DEVICES AND THE EFFECTIVENESS OF HYDROGEN PEROXIDE GAS PLASMA AGAINST *BACILLUS SUBTILIS* SPORES

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ABSTRACT

OBJECTIVES: To determine the microbial load found on used critical medical devices (5 spinal anesthesia needles, 21 catheters, and 28 sheaths) prior to sterilization and to evaluate the effectiveness of hydrogen peroxide gas plasma against inoculated *Bacillus subtilis* var *globigii* (American Type Culture Collection 9372) spores.

METHODS: Membrane filter and pour-plate methods were applied to estimate total microbial loads (aerobic and anaerobic, mesophilic and thermophilic, vegetative and spore forms). Spinal anesthesia needles (102 units) and sheath components (61 units) were inoculated with a suspension of *B subtilis* spores. After drying, the devices were sterilized with hydrogen peroxide gas plasma.

RESULTS: Higher counts of aerobic, mesophilic, and fungal organisms were recovered when the drying period was insufficient. Anaerobic spores were not found in any analyzed presterilization items. The hydrogen peroxide gas plasma effected a 5 to 7 log_{10}-fold reduction in *B subtilis* spore counts in well-dried needles and sheath components.

CONCLUSIONS: The success of hydrogen peroxide gas plasma sterilization depends mostly on educating the staff to assure well-cleaned and dried reusable medical devices, allowing penetration of the hydrogen peroxide gas plasma into the critical points of the items and providing a reduction in organisms (*Infect Control Hosp Epidemiol* 1999;20:465-472).

Invasive devices are essential in modern medical practice. However, the use of intravenous items is limited by a range of possible local or systemic infections. Critical items that enter sterile tissue or the vascular system carry the highest risk of infection.[1]

The cost of care involving critical medical devices could be reduced substantially if the devices could be reused. However, articles that possess narrow lumens or fissures, or that cannot be disassembled, present challenges to cleaning, drying, and sterilization.

Angiographic catheters, spinal anesthesia needles, and sheath introducers used to perform mechanical functions are considered critical items. Acceptable sterility levels can be achieved by standard procedures even when the devices are intentionally contaminated.[2-4] In a survey conducted by *Infection Control & Sterilization Technology,* 35% of responding hospitals reported the reuse of "single-use only" devices, primarily catheters, blades, and knives.[5] The extent to which labeled single-use–only medical devices may be reused while maintaining cleanliness, sterility, and structural integrity has been widely debated in Australia,[6] and studies in Canada have proved the viability of reusable angiographic catheters.[7]

To achieve an acceptable sterility assurance level through a terminal sterilization process,[8] the critical medical device must be freed from all adherent materials that interfere with the sterilizing agent.

This study seeks to estimate the presterilization microbial load on critical medical devices (spinal anesthesia needles, sheath introducers, and catheters) after hospital cleaning and drying procedures and to evaluate the effectiveness of hydrogen peroxide gas plasma sterilization against *Bacillus subtilis* spores inoculated into medical devices.

METHODS

Overview

Dehydrated plate count agar (PCA), tryptic soy broth (TSB), and Sabouraud dextrose agar culture media (Difco, Detroit, MI) were prepared following the manufacturer's directions and were steam sterilized (121°C/15 min/L) prior to use.

Prior to inoculation with *B subtilis* suspension spores, medical devices were cleaned, dried, wrapped in Tyvek-Mylar pouches (Advanced Sterilization Products,

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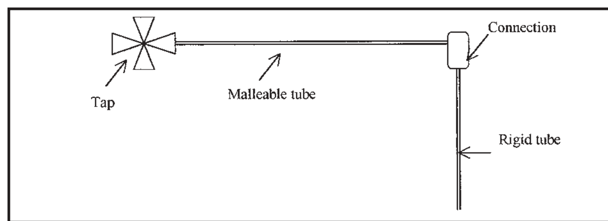

FIGURE. Diagrammatic representation of sheath and draining components.

Johnson & Johnson, Arlington, TX), and sterilized by hydrogen peroxide gas plasma (Sterrad 100, Advanced Sterilization Products, Johnson & Johnson, Irvine, CA). Tests were performed in a laminar airflow class 100 cabinet (Veco, VLFS-12M, Campinas, SP, Brazil).

The estimation of the microbial load in medical devices was performed in three stages[9,10]: (1) removal of the groups of microorganisms that either survived the cleaning procedure at the hospital or, for inoculated *B subtilis* spores, survived the sterilization process; (2) transfer of those microorganisms into optimal cultural conditions; and (3) a count of the groups of microorganisms that were removed.

Presterilization Microbial Load: Medical Devices

Medical devices evaluated 5 spinal anesthesia stainless steel needles (100-mm×0.6-mm), 21 angiographic catheters (100-cm length×1.0-mm width), and 28 sheath introducers. They were used in regular practice, submitted to hospital cleaning and drying procedures, wrapped in Tyvek-Mylar pouches, and stored overnight at room temperature. The interval from cleaning and drying of the devices at the hospital and microbiological analysis at the São Paulo University laboratory was 24 hours. The items were rinsed with physiologic solution (0.85% wt/vol), which was submitted immediately for microbiological analysis. The inner walls of the needle were rinsed five times with 20-mL volumes of physiologic solution, using a syringe. The total volume obtained of 100 mL was homogenized in an incubator at 200 rpm for 30 minutes at 25°C (Tecnal TE-420, Piracicaba, SP, Brazil). The inner walls of the catheter were rinsed 10 times with 10-mL volumes of physiologic solution (100 mL total), using a syringe. The rinsing catheter then was reduced to 2-cm cylinders and mixed (200 rpm, 30 min, 25°C) with 100 mL of physiologic solution. With the aid of a disposable syringe, the components of the sheath introducer were rinsed with 100 mL of physiologic solution, which was homogenized (200 rpm, 30 min, 25°C). The draining components then were detached from the sheath (Figure)—tap, connection, malleable plastic and rigid tubes (reduced to 1-cm cylinders)—and mixed (200 rpm, 30 min, 25°C) with 100 mL of physiologic solution.

Microbiological Tests

Colony counts were estimated, using standard procedures for evaluation of water,[11] for the following groups of microorganisms: total aerobic, anaerobic, mesophilic, and thermophilic bacteria, including spore formers and fungi. The 100 mL of the physiologic solution taken from rinsing catheters and sheaths was subdivided into samples of 20 mL and filtered through a sterilizing membrane (47-mm diameter×0.45-µm pores, Millipore, Belford, MA). Subsequently, the membrane was laid on the PCA surface. The plates were incubated in aerobic and anaerobic conditions for 48±3 hours at 35°C and 62°C, respectively, for total counts of mesophilic and thermophilic organisms. A volume of 20 mL of the rinsing-needle physiologic solution was serially diluted in TSB. One-mL samples of each dilution were seeded into PCA through the pour-plate method and incubated as above. For the count of spore-form bacteria, 5-mL volumes of rinsing-device physiologic solutions were transferred to tubes. One tube was heated to 80°C for 10 minutes (mesophilic spores) and the other to 100°C for 20 minutes (thermophilic spores). After cooling, the solutions were seeded into PCA through the pour-plate method and incubated as above. The count of fungi was done through serial dilution of the rinsing-device physiologic solutions in Sabouraud dextrose agar by the pour-plate method, and the plates were incubated at 25°C for 7 days. Colonies were counted on selected plates and were reported as the average number of colony-forming units (CFU) per medical device.

If no organisms were detected on plate counts, and the item incubated in broth showed no growth, then the item was considered sterile. If there was growth only in the TSB from the incubated item, but no recorded plate counts, this represented between one and five residual organisms on the item (the limit of detection on the quantitative plates was five organisms per unit).

Sterilization of Inoculated **B subtilis** *Spores*

Product inoculation involves artificial contamination of a sterile product with a known number of microorganisms, usually bacterial spores. By exposing the product to a system being validated, the fraction of spores removed can be estimated. A relation between the sterilization system variables and the medical device complexity may be established and provided with a list of sterilization equipment uses.[8,12]

A suspension of *B subtilis* spores was inoculated into 102 stainless steel spinal anesthesia needles, 12 sheaths, 14 detached sheath taps, and 13 detached sheath connections, which then were dried and submitted to hydrogen peroxide gas plasma sterilization.

Microorganism and Inoculation of Medical Devices

Cultures of *B subtilis* var *globigii* (American Type Culture Collection [ATCC], 9372) developed at 35°C for 24 hours on an inclined 7-cm–width PCA surface (seven tubes) were suspended in 14 mL of physiologic solution. The homogenized suspension was transferred to a 200-mL inclined PCA surface in a Roux bottle and incubated at 35°C for 7 days. At the time of maximum sporulation (6

TABLE 1
COLONY COUNTS OF ORGANISMS DETECTED ON USED SPINAL ANESTHESIA STAINLESS STEEL NEEDLES (100 MM×0.6 MM) FOLLOWING ROUTINE HOSPITAL CLEANING AND DRYING

	Aerobic (CFU/Needle)					Anaerobic (CFU/Needle)			
	Mesophilic		Thermophilic			Mesophilic		Thermophilic	
Needle	Total	Spore Former	Total	Spore Former	Fungi	Total	Spore Former	Total	Spore Former
1	1.8×10^2	<5*	<5	<5	<5	<5	<5	<5	<5
2	4.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
3	<5	<5	<5	<5	<5	<5	<5	<5	<5
4	<5	<5	<5	<5	<5	<5	<5	<5	<5
5	<5	<5	<5	<5	<5	<5	<5	<5	<5

Abbreviations: CFU, colony-forming units.
* Lower limit of detection.

days), as determined by daily phase contrast microscopy, the developed spore culture was suspended and mixed (1,060 rpm for 15 min) in 100 mL of calcium acetate (0.02 M). The final pH of the suspension was adjusted to 9.7 with 0.14% (wt/vol) calcium hydroxide ($Ca(OH)_2$) solution and centrifuged four times at 1,935*g* for 30 minutes. After each centrifugation, the supernatant was decanted, and the spore pellets were washed by suspending them in cooling 0.02M calcium acetate solution (pH=9.7) and adjusted to pH 9.7 with 0.14% calcium hydroxide solution. The final spore suspension (microscopically assessed for the absence of vegetative forms) was stored at 4°±1°C.[13]

Prior to inoculation, the *B subtilis* suspension was diluted in calcium acetate (0.02M) to 10^6 spores per 0.01 mL. Approximately 28 µL of the suspension was injected in each needle. The item was kept horizontal at 45°C for 72 hours in a mechanical aerator oven to dry residual water, causing the spores to adhere along the edge of the inner wall. The needles were individually packed into unsealed Tyvek-Mylar. Samples 1 through 10 were stored in a refrigerator at 8°C up to 72 hours. Samples 11 through 74 were stored at 25°C for 72 hours in a mechanical aerator incubator. Samples 75 through 102 were gently freed by pulling a wire guide through the lumen to ensure that spore clumps did not obstruct the gas plasma penetration. The lumens were flushed with dried air, and the needles were stored at 25°C for 72 hours.

Approximately 40 µL of the suspension was introduced into each critical point of 12 sheaths and 27 detached components: tap entrances, connections, and malleable plastic tubes (through an open hole—25 mm×0.8 mm—afterwards glued with silicon). All sheaths and detached components were kept horizontal and dried at 45°C for 72 hours.

Hydrogen Peroxide Gas Plasma Sterilization System

The Sterrad 100 alternative low-temperature sterilization system combines radio waves with hydrogen peroxide to create a gas plasma; these free radicals interact with and destroy microorganisms.[14] This has been used in Brazilian hospitals with 300 to 1,200 monthly surgical procedures.[15]

The dried inoculated items were conditioned in Tyvek-Mylar pouches and sterilized within the process chamber (55-cm diameter×100-cm depth) of the Sterrad 100 sterilizer. The 75-minute operational cycle at 45°C was programmed in five stages[15]: (1) vacuum for 5 to 20 minutes at a pressure of 300 mTorr; (2) injection of 1.8 mL (58% vol/vol) of hydrogen peroxide by breaking a cassette ampoule, reaching a 12-torr maximum pressure over 6 minutes; (3) steam diffusion for 42 minutes; (4) plasma generation by radio-frequency energy (13.56 MHz) at 500 mTorr pressure for 15 minutes; and (5) ventilation for 4 minutes. Cycle efficiency was evaluated with a self-contained bioindicator system (10^6 spores of *B subtilis* var *globigii* ATCC 9372 per inoculated unit) and a chemical indicator.

Determination of the Number of Surviving Spores

In each group of inoculated devices, one of the specimens was used as a positive control to count *B subtilis* spores. The inner wall of a needle was rinsed 10 times with 5 mL of TSB. The count of the surviving spores was done by serial dilution of 2 mL of TSB through the pour-plate method in PCA, and the plates were incubated at 35°C for 24 to 48 hours. Surviving spores were confirmed by incubation of the remaining 3 mL of TSB at 35°C for 7 days. To achieve a positive growth, 3 mL of TSB were thermally shocked at 80°C for 10 minutes, cooled, and seeded by loop transfer into a sterilized, 5-mL TSB tube and incubated at 35°C for 7 days. Each component was detached from the sheath unit, and the malleable plastic tube was reduced to 1-cm cylinders. Each detached component was transferred to a 50-mL TSB flask, the system was magnetically mixed for 30 minutes, and incubated at 35ºC for 7 days. To get growth, the 5 mL of TSB was thermally shocked at 80°C for 10 minutes, cooled, seeded by loop transfer into a sterilized 5-mL TSB tube, and incubated at 35°C for 7 days.

RESULTS

Microbial Load (Bioburden)

The presterilization microbial load for medical devices, following hospital cleaning and drying proce-

TABLE 2
COLONY COUNTS OF ORGANISMS DETECTED ON USED ANGIOGRAPHIC CATHETERS (100-CM LENGTH×1.0-MM DIAMETER)

	Aerobic (CFU/Catheter)					Anaerobic (CFU/Catheter)			
	Mesophilic		Thermophilic			Mesophilic		Thermophilic	
Needle	Total	Spore Former	Total	Spore Former	Fungi	Total	Spore Former	Total	Spore Former
1	>1.5×10^3	5.5×10^1	<5	<5	5.3×10^3	3.5×10^1	<5	<5	<5
2	<5	2.0×10^1	<5	<5	2.0×10^2	1.0×10^1	<5	<5	<5
3	1.1×10^2	5.0×10^0	2.0×10^2	<5	<5	3.5×10^1	<5	<5	<5
4	3.0×10^2	1.0×10^1	<5	<5	3.3×10^6	<5	<5	<5	<5
5	4.0×10^1	5.0×10^0	<5	<5	<5	<5	<5	<5	<5
6	>1.5×10^3	<5	<5	<5	2.5×10^4	<5	<5	<5	<5
7	5.0×10^0	<5	<5	<5	<5	<5	<5	<5	<5
8	1.0×10^1	1.0×10^1	<5	<5	<5	<5	<5	<5	<5
9	<5	<5	<5	<5	<5	<5	<5	<5	<5
10	>1.5×10^3	5.0×10^0	<5	<5	4.6×10^3	<5	<5	<5	<5
11	1.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
12	1.8×10^2	<5	<5	<5	<5	<5	<5	<5	<5
13	<5	<5	<5	<5	<5	<5	<5	<5	<5
14	<5	<5	<5	<5	<5	<5	<5	<5	<5
15	1.0×10^1	<5	<5	<5	4.0×10^2	<5	<5	<5	<5
16	5.0×10^0	<5	<5	<5	<5	<5	<5	<5	<5
17	2.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
18	<5	<5	<5	<5	<5	<5	<5	<5	<5
19	<5	<5	<5	<5	<5	<5	<5	<5	<5
20	3.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
21	<5	<5	<5	<5	<5	<5	<5	<5	<5

Abbreviation: CFU, colony-forming units.

dures, is presented in Tables 1, 2, and 3. The cleaning operations guaranteed almost total removal of encrusted material from the needles. Mesophilic microorganisms were detected at 1 to 2 logs in only two of five needles (Table 1). There was no growth of any of the other organism groups.

The bioburden estimated for 100-cm tubing catheters with 1.0-mm narrow lumens (Table 2) showed growth of aerobic mesophilic organisms in 14 of 21 analyzed units; the count exceeded 10^3 CFU in units 1, 6, and 10. Anaerobic mesophilic organisms were found in the first three catheters at a maximum level of 3.5×10^1 CFU, indicating the possible presence of encrusted residual organic material. After instructing hospital staff about the importance of removing all organic residues from the lumen, anaerobic organisms of any type were no longer found in any further samples.

Spore-forming aerobic mesophilic organisms were confirmed in 7 of 21 units, at a maximum level of 5.5×10^1 CFU. The aerobic thermophilic group was found only in sample 3, at a level of 10^2 CFU.

Fungi were found in units 1, 2, 4, 6, 10, and 15, at a maximum level of 3.3×10^6 CFU. This suggested the presence of moisture, which allowed the possible proliferation of polluting microorganisms inside the catheters prior to sterilization. After specimen 10, hospital staff were reminded of the importance of the drying operation to inhibit the development of opportunist contaminants, and only one further specimen was positive for fungi.

The sheaths are more complex devices, and cleaning and drying are more difficult; 17 of 28 units showed growth of mesophilic aerobes (Table 3). Units 2, 3, 5, 7, 8, 18, 19, 26, and 27 showed no growth of microorganisms (ie, less than 5 CFU/unit), indicating proper cleaning and drying procedures. Anaerobic growth was detected (at a level of 1.5×10^1 CFU) only in unit 25. Growth of thermophilic organisms was not detected in any of the 28 units. Fungi were detected in 5 samples, with counts exceeding 10^7 CFU in unit 24.

Sterilization of Inoculated **B subtilis** *Spores*

The effectiveness of the hydrogen peroxide gas plasma sterilizer in the reduction of inoculated *B subtilis* spores in the sterile lumens of needles and sheath introducers is shown in Tables 4 and 5, respectively.

Needles 1 through 10 were dried at 45°C for 72 hours, packed in unsealed Tyvek-Mylar pouches, and thereafter refrigerated up to 72 hours prior to sterilization. After refrigeration, humidity condensation was observed inside the package and therefore inside the needle. The condensation of residual humidity interfered with the efficiency of the sterilant gas plasma. This was caused by the interaction of water and hydrogen peroxide.

Needles 11 through 74 were dried, packed, and

TABLE 3
COLONY COUNTS OF ORGANISMS DETECTED ON USED SHEATH INTRODUCERS

	Aerobic (CFU/Sheath)					Anaerobic (CFU/Sheath)			
	Mesophilic		Thermophilic			Mesophilic		Thermophilic	
Needle	Total	Spore Former	Total	Spore Former	Fungi	Total	Spore Former	Total	Spore Former
1	7.9×10^4	<5	<5	<5	1.3×10^4	<5	<5	<5	<5
2	<5	<5	<5	<5	<5	<5	<5	<5	<5
3	<5	<5	<5	<5	<5	<5	<5	<5	<5
4	6.0×10^5	<5	<5	<5	<5	<5	<5	<5	<5
5	<5	<5	<5	<5	<5	<5	<5	<5	<5
6	<5	2.2×10^4	<5	<5	<5	<5	<5	<5	<5
7	<5	<5	<5	<5	<5	<5	<5	<5	<5
8	<5	<5	<5	<5	<5	<5	<5	<5	<5
9	7.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
10	<5	2.8×10^4	<5	<5	<5	<5	<5	<5	<5
11	2.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
12	1.6×10^3	<5	<5	<5	<5	<5	<5	<5	<5
13	3.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
14	4.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
15	5.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
16	1.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
17	5.0×10^0	<5	<5	<5	<5	<5	<5	<5	<5
18	<5	<5	<5	<5	<5	<5	<5	<5	<5
19	<5	<5	<5	<5	<5	<5	<5	<5	<5
20	1.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
21	1.0×10^1	<5	<5	<5	<5	<5	<5	<5	<5
22	1.5×10^1	<5	<5	<5	<5	<5	<5	<5	<5
23	1.5×10^3	<5	<5	<5	1.5×10^4	<5	<5	<5	<5
24	$>1.5\times10^3$	<5	<5	<5	$>3.0\times10^7$	<5	<5	<5	<5
25	$>1.5\times10^3$	<5	<5	<5	7.9×10^4	<5	<5	1.5×10^1	<5
26	<5	<5	<5	<5	<5	<5	<5	<5	<5
27	<5	<5	<5	<5	<5	<5	<5	<5	<5
28	$>1.5\times10^3$	<5	<5	<5	3.0×10^2	<5	<5	<5	<5

Abbreviation: CFU, colony-forming units.

stored prior to sterilization at 25°C, thus avoiding water condensation. However, these needles had varying results, with sterilization confirmed in 36 of 64 inoculated needles (11-35, 40, 42-44, 46, 48, 51, 53-55, 61; Table 4). This variability was thought to be due to nonstandardized drying conditions.

Thereafter, needles 75 through 102 were inoculated with a maximum count of 10^7 spores per unit and dried at 45°C for 72 hours. Their lumens were freed with guide wire, flushed with dried air, and then packed and stored at 25°C for 72 hours. It was confirmed that the recoverable bioburden after clearing the obstruction remained essentially the same as the initial inoculum and needle (data not shown). Of these 28 needles, only 3 showed growth.

Needle 86 retained 9.2×10^2 viable spores. There were no colonies on the plate counts for needles 85 and 93, but there was growth in the TSB, indicating that there were between one and five organisms remaining in the needles.

The survival of inoculated *B subtilis* spores after processing with hydrogen peroxide gas plasma is shown in Table 5 (the difference of approximately a half-log inoculum for the attached and detached components is the range suggested by the *United States Pharmacopeia*).[12]

The reduction of *B subtilis* spores by at least 6 $\log_{10}$, after inoculation into the most inaccessible points of attached sheath components, was confirmed for 5 of 9 taps, 9 of 11 malleable plastic tubes, and 10 of 11 connections (Table 5). In contrast, a reduction by at least 5 $\log_{10}$ was confirmed after sterilization of the detached components for 0 of 13 taps and 2 of 12 connections. Although these results appear counterintuitive, they are explained by the fact that, in the first experiments, the piston within the tap was kept in a position to favor the flow of gas through the tap and into the rest of the attached sheath components. When the tap was sterilized separately, the piston was left in a position that obstructed the penetration of the gas into the critical parts of the tap itself. Thus, the position of the pistons within the taps appeared to be the key factor determining success of the sterilization procedure.

TABLE 4
SURVIVAL OF *BACILLUS SUBTILIS* SPORES INOCULATED INTO STAINLESS STEEL SPINAL ANESTHESIA NEEDLES AFTER PROCESSING IN THE HYDROGEN PEROXIDE GAS PLASMA SYSTEM*

Needle	Length× Diameter (mm)	Initial (CFU/Needle)	Survival (CFU/Needle)
1	100×0.6	6.7×10^{5}	1.0×10^{1}
2	100×0.6	6.7×10^{5}	3.0×10^{2}
3	100×0.6	6.7×10^{5}	1.0×10^{1}
4	100×0.6	6.7×10^{5}	7.0×10^{1}
5	100×0.6	6.7×10^{5}	5*
6	100×0.6	6.7×10^{5}	$>3.0\times10^{2}$
7	100×0.6	6.7×10^{5}	1.5×10^{1}
8	100×0.6	6.7×10^{5}	$>3.0\times10^{2}$
9	100×0.6	6.7×10^{5}	$>3.0\times10^{2}$
10	100×0.6	6.7×10^{5}	5
11	8.89×0.4	8.7×10^{5}	<5
12	8.89×0.4	8.7×10^{5}	<5
13	8.89×0.4	8.7×10^{5}	<5
14	8.89×0.4	8.7×10^{5}	<5
15	100×0.6	4.0×10^{5}	<5
16	100×0.6	4.0×10^{5}	<5
17	100×0.6	4.0×10^{5}	<5
18	100×0.6	4.0×10^{5}	<5
19	100×0.6	4.0×10^{5}	<5
20	100×0.6	4.0×10^{5}	<5
21	100×0.6	4.0×10^{5}	<5
22	100×0.6	4.0×10^{5}	<5
23	100×0.6	4.0×10^{5}	<5
24	100×0.6	4.0×10^{5}	<5
25	100×0.6	4.0×10^{5}	<5
26	100×0.6	4.0×10^{5}	<5
27	100×0.5	4.0×10^{5}	<5
28	100×0.5	4.0×10^{5}	<5
29	100×0.5	4.0×10^{5}	<5
30	100×0.8	4.0×10^{5}	<5
31	8.89×0.4	4.0×10^{5}	<5
32	8.89×0.4	4.0×10^{5}	<5
33	8.89×0.4	4.0×10^{5}	<5
34	8.89×0.4	4.0×10^{5}	<5
35	100×0.6	3.4×10^{6}	<5
36	70×0.6	3.4×10^{6}	$>3.0\times10^{2}$
37	70×0.6	3.4×10^{6}	5
38	70×0.6	3.4×10^{6}	8.0×10^{1}
39	70×0.6	3.4×10^{6}	$>3.0\times10^{2}$
40	70×0.6	3.4×10^{6}	<5
41	70×0.6	3.4×10^{6}	5
42	100×0.6	2.9×10^{6}	<5
43	100×0.6	2.9×10^{6}	<5
44	100×0.6	2.9×10^{6}	<5
45	100×0.6	2.9×10^{6}	5
46	100×0.6	2.9×10^{6}	<5
47	100×0.5	2.3×10^{5}	5
48	100×0.5	2.3×10^{5}	<5
49	100×0.5	2.3×10^{5}	5
50	100×0.5	2.3×10^{5}	5
51	100×0.5	2.3×10^{5}	<5
52	100×0.5	2.3×10^{5}	5
53	100×0.5	2.3×10^{5}	<5
54	100×0.5	2.3×10^{5}	<5
55	100×0.5	2.3×10^{5}	<5
56	100×0.5	2.3×10^{5}	5
57	100×0.5	2.3×10^{5}	5
58	70×0.6	2.3×10^{5}	5
59	70×0.6	2.3×10^{5}	5
60	70×0.6	2.3×10^{5}	5
61	70×0.6	2.3×10^{5}	<5
62	70×0.6	2.3×10^{5}	5
63	100×1.2	1.4×10^{8}	3.7×10^{4}
64	100×1.2	1.4×10^{8}	1.5×10^{4}
65	100×1.2	1.4×10^{8}	5
66	100×1.2	1.4×10^{8}	3.3×10^{4}
67	100×0.8	8.3×10^{7}	$>3.0\times10^{5}$
68	100×0.8	8.3×10^{7}	5
69	100×0.8	8.3×10^{7}	$>3.0\times10^{5}$
70	100×0.8	8.3×10^{7}	$>3.0\times10^{5}$
71	100×1.2	2.5×10^{7}	1.0×10^{4}
72	100×1.2	2.5×10^{7}	4.6×10^{2}
73	100×1.2	2.5×10^{7}	5.7×10^{2}
74	100×1.2	2.5×10^{7}	5
75	100×0.8	1.0×10^{7}	<5
76	100×0.8	1.0×10^{7}	<5
77	100×0.8	1.0×10^{7}	<5
78	100×0.8	1.0×10^{7}	<5
79	100×1.2	3.0×10^{7}	<5
80	100×1.2	3.0×10^{7}	<5
81	100×1.2	3.0×10^{7}	<5
82	100×1.2	3.0×10^{7}	<5
83	100×0.8	2.2×10^{7}	<5
84	100×0.8	2.2×10^{7}	<5
85	100×0.8	2.2×10^{7}	5
86	100×0.8	2.2×10^{7}	9.2×10^{2}
87	100×1.2	8.7×10^{7}	<5
88	100×1.2	8.7×10^{7}	<5
89	100×1.2	8.7×10^{7}	<5
90	100×1.2	8.7×10^{7}	<5
91	100×0.8	9.7×10^{7}	<5
92	100×0.8	9.7×10^{7}	<5
93	100×0.8	9.7×10^{7}	5
94	100×0.8	9.7×10^{7}	<5
95	100×1.2	1.67×10^{7}	<5
96	100×1.2	1.67×10^{7}	<5
97	100×1.2	1.67×10^{7}	<5
98	100×1.2	1.67×10^{7}	<5
99	100×0.8	1.53×10^{7}	<5
100	100×0.8	1.53×10^{7}	<5
101	100×0.8	1.53×10^{7}	<5
102	100×0.8	1.53×10^{7}	<5

Abbreviation: CFU, colony-forming units.
* Needles 11-14 and 35-41 had a plastic connection end. The needles were dried for 72 hours at 45°C, flushed with air (75-102), and stored for 72 hours prior to sterilization: 1-10 at 8°C, 11-102 at 25°C. Confirmed growth in tryptic soy broth with new growth on plate was reported as 5 colony-forming units/needle.

DISCUSSION

The relation between the bioburden on a product and the post-sterilization recovery of microorganisms may depend on article configuration; materials used, such as steel for needles, polymers for catheters and sheaths; integrity of the article's structure after repeated exposure to sequential steps of regular use, cleaning, and drying procedures; and sterilizer operation.

The worse case of presterilization bioburden on used medical devices submitted for reprocessing would be the presence of bacteria spore forms. Mesophilic spore forms were detected on 7 of 21 catheters and 2 of 28 sheath components, and thermophilic spore forms were found on 1 of 21 catheters and 1 of 28 sheath components after cleaning. It is likely that the spores were introduced when the medical devices were being cleaned or rinsed and that adequate humidity during storage allowed overgrowth of these "environmental" organisms. As such, the cleaning and drying procedures may critically alter the presterilization microbial load on reprocessed medical devices. This demonstrates that it is important to consider not only the microbial load acquired from patient contact but also that overgrowth of environmental organisms acquired during reprocessing may be a critical consideration.

The adherence of contaminating microorganisms varies considerably, as does the effectiveness of cleaning techniques in removing microorganisms from the material. The cleaning and drying procedures of medical devices influence the efficacy of the gas plasma sterilization system. Particle deposits and residual moisture complicate the penetration of hydrogen peroxide gas plasma into the lumen of the article, altering microbial destruction. Water, cellulose, and encrusted mixed particles absorb hydrogen peroxide in the vapor-injection stage, thus lowering hydrogen peroxide gas plasma access to the microbial load and consequent destructive effects.

The accepted cleaning and drying assurance level is a reduction in the microbial counts by 5-$\log_{10}$ units.[8] A testing procedure that can be standardized and permits quantitative evaluation must be required for cleaning and also for drying. *B subtilis* spores could be the test organism used for demonstrating bactericidal efficiency.

Cleaning water and drying air might be considered sources of many sorts of microorganisms. Maintenance of a good hygienic standard for water and air prevents cleaning and drying from contaminating medical devices. Tap water and air should be from suitable sources and preferably filtered before being applied to medical devices, but need not necessarily be sterile. The air also should be dried, without any residual moisture.

According to Alfa,[16] a recent survey indicated that 16 (23%) of 71 gastrointestinal endoscope samples had more than 10^5 CFU of bacteria immediately after cleaning and disinfection procedures were completed. The long thin lumens of flexible endoscopes make the cleaning and disinfecting procedures difficult.

Alfa and Sitter[17] rinsed 10 mL of fluid through used endoscopes prior to washing and disinfection, and detected from 6×10^4 CFU/mL in bronchoscopes to 5×10^5 CFU/mL in colonoscopes. They observed that, although the bacterial load might be reduced by 1 to 2 logs by the cleaning procedure and 2 to 3 logs by sterilizers in the presence of serum and salt, the margin of safety would be very narrow.

TABLE 5
SURVIVAL OF INOCULATED *BACILLUS SUBTILIS* SPORES FOLLOWING HYDROGEN PEROXIDE GAS PLASMA PROCESSING (INITIAL POPULATION: 1-3×10^6 SPORES PER ATTACHED SHEATH COMPONENT AND OF 4.4-9.7×10^5 PER DETACHED SHEATH COMPONENT)

	Negative/Total	(%)
Attached components		
Tap	5/9	55.6
Tube	9/11	81.8
Connection	10/11	90.9
Detached components		
Tap	0/13	0
Connection	2/12	16.7

Alfa[16] and Alfa et al[18] demonstrated difficulty in achieving sterilization of narrow lumens in the presence of serum and salt. Neither plasma sterilizers (Abtox, Mundelein, IL, and Sterrad) nor ethylene oxide (12/88 and 100%) appear to be effective methods for ensuring the expected sterility when serum and salt are present. Other researchers have demonstrated similar difficulty for plasma sterilizers in reducing bacteria by 6 $\log_{10}$ on narrow lumens in the presence of blood or salt. Alfa et al[18] confirmed that, in the absence of any protein or salt challenge, the sterilizers are able to sterilize superficial microorganisms adequately even through narrow-lumen (3.2-mm internal diameter) tubing of 125-cm length.

The inoculated and well-dried narrow-lumen needles and difficult-to-penetrate sheath components provided free gas plasma penetration to their inner surfaces and achieved a sterility assurance level of 10^{-6} in reference to *B subtilis* spores. The cleaning and drying procedures are essential to provide the catheters (1.0-mm width) with an easy gas diffusion through their 100-cm length.

The success of the hydrogen peroxide gas plasma sterilizer depends mostly on educating hospital staff how to clean and dry reused medical devices. This will help ensure penetration of the hydrogen peroxide plasma into the critical areas of the medical devices, thereby facilitating the desired 10^{-6} microbial load reduction.

REFERENCES

1. Rutala WA. Disinfection and sterilization of patient-care items. *Infect Control Hosp Epidemiol* 1996;17:377-384.
2. Bentolita P, Jacob R, Roberge F. Effects of re-use on the physical characteristics of angiographic catheters. *J Med Eng Technol* 1990;4:254-259.
3. Jacobson JA, Schwartz CE, Marshall HW, Conti M, Burke JP. Fever, chills and hypotension following cardiac catheterization with single- and multiple-use disposable catheters. *Cathet Cardiovasc Diagn* 1983;9:39-46.
4. Ravin CE, Koehler PR. Reuse of disposable catheters and guide wires. *Radiology* 1977;122:577-579.
5. Booth AF. The manufacturer's role in safe reuse. *Medical Device & Diagnostic Industry* 1997;87:127-134.

6. Collignon P J, Graham E, Dreimanis DE. Reuse in sterile sites of single-use medical devices: how common is this in Australia? *Med J Aust* 1996;164:533-536.
7. Comis J. Reusing Single-Use Cardiac Catheters. *Dimensions* 1991;October: 32-34.
8. Association for the Advancement of Medical Instrumentation. *Design, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A Guide for Device Manufacturers.* Report no. 12. Arlington, VA: AAMI; 1995.
9. Morrissey RF. Bioburden: a rational approach. In: Gaughran ERL, Morrissey RF, eds. *Sterilization of Medical Products, vol. II.* 2nd ed. Montreal, Quebec, Canada: Multiscience Publications; 1981:11-33.
10. Morrissey RF, Phillips CB. *Sterilization Technology: A Practical Guide for Manufacturers and Users of Health Care Products.* New York, NY: VanNostrad Reinhold; 1993:518.
11. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater.* 19th ed. Washington, DC: APHA; 1995:9-31, 9-38.
12. *United States Pharmacopeia.* 23rd ed. Rockville, MD: United States Pharmacopeial Convention; 1995:1847-1849, 1976-1981.
13. Penna TC, Machoshvili IA, Taqueda ME. Thermal resistance of *Bacillus stearothermophilus* spores on strips previously treated with calcium. *PDA J Pharm Sci Technol* 1996;50:227-237.
14. Ferraz CA. STERRAD opte pelo futuro. *Enfoque* 1995;21:12-15.
15. Cassola MA, Ishsaki E, Ferraz CA, Vessoni Penna TC. Esterilização de artigos médico hospitalares por plasma de peróxido de hidrogênio. *Laes & Haes* 1997;19:88-100.
16. Alfa MJ. Plasma-based sterilization. The challenge of narrow lumens. *Infection Control & Sterilization Technology* 1996;19-24.
17. Alfa MJ, Sitter DL. In-hospital evaluation of orthophthaladehyde as a high-level disinfectant for flexible endoscopes. *J Hosp Infect* 1994; 26:15-56.
18. Alfa MJ, DeGagne P, Olson N, Puchalski T. Comparison of ion plasma, vaporized hydrogen peroxide, and 100% ethylene oxide sterilizers to the 12/88 ethylene oxide gas sterilizer. *Infect Control Hosp Epidemiol* 1996;17:92-100.