Safety and Cleaning of Medical Materials and Devices

Katharine Merritt, Victoria M. Hitchins, Stanley A. Brown

FDA, Center for Devices and Radiological Health, Division of Life Sciences, HFZ 112, Rockville, Maryland 20852

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Abstract: A study was undertaken to evaluate different procedures to safely remove microorganisms, protein, and mammalian cells from materials and provide a suitable method for cleaning and assessing effectiveness of cleaning medical devices for reuse or for analysis of failure. Safety considerations for the personnel performing the cleaning or handling the device after cleaning are important issues. Polystyrene plates (96 well) were used to simulate device surfaces not amenable to manual scrubbing. *Staphylococcus epidermidis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa,* and oral flora were grown in the plates. The plates were stained with crystal violet and the optical densities recorded. The results indicated that *E. coli* did not adhere well and *Pseudomonas* formed clumps that were easily detached from the surface of the plates. However, *S. epi, C. albicans,* and the oral organisms formed adherent biofilms that were difficult to remove from the plates. Detergents with enzymes and sodium hypochlorite (NaOCl) bleach were both effective in removing the biofilm. Other detergents and surfactants were not effective. The aldehyde agents did not remove the organisms and made further cleaning difficult. Allowing the biofilm to dry first made cleaning very difficult. Only the NaOCl bleach could subsequently remove the dried or aldehyde fixed organisms from the wells. The same 96-well polystyrene plate format was used to measure the amount of protein and cell adherence as well as the effectiveness of subsequent cleaning. Bradford reagent was used to detect protein as a measure of the cleaning efficacy. As with the bacteria, NaOCl bleach was effective at removing the protein and cells that had been dried or fixed by formalin or alcohol, whereas detergent with enzymes was not very effective. This study confirmed that used medical devices, contaminated with microorganisms, protein, and/or mammalian cells, should not be allowed to dry before cleaning and that a thorough cleaning procedure should precede sterilization or disinfection (with the exception of NaOCl bleach which also cleans). © 2000 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 53: 131–136, 2000

INTRODUCTION

During the last quarter century, the practice of medicine has moved from the use of (a) items that were cleaned, sterilized, and reused; to (b) single use disposable items; and now to (c) the reuse of disposable single use items that have been cleaned and reprocessed.[1–3] The need to control costs has prompted the reuse of items that were manufactured originally for single use and then disposal.[4] Thus, there is a mix of items being used. Those that are designed to be cleaned and reused, single use devices that are discarded after use, and single use devices that are designed to be discarded after a single use but are being cleaned and reused on the same patient or on different patients.[1–4]

There are three basic safety concerns with reusing medical devices. *First* is the cleanliness and sterility of the device and the subsequent safety to the patient, which also includes issues of cleanliness affecting subsequent the disinfection or sterilization process. *Second* is the effect of cleaning, disinfection, and sterilization on the chemical, physical, and mechanical integrity of the devices. The *third* concern is providing for the safety of the personnel who are responsible for cleaning, disinfecting, or resterilizing the devices. The issue of safe and effective decontamination of devices is also important in device retrieval and analysis.

This study was undertaken to determine the efficacy of various methods used to clean device materials. This brief report describes cleaning clinically relevant microorganisms, protein, and mammalian cells from 96-well polystyrene plates. Although polystyrene is not among the device materials currently being reused, the 96-well format allows quantitative assessment of effectiveness of removing organic materials from surfaces. The results from this study provide data on protocols for cleaning medical device materials for reuse of devices or for analysis of retrieved devices. Sensitive dye techniques are used to assess cleaning, which is defined as the removal of detectable extraneous material.

Correspondence to: Katharine Merritt, 17704 Stoneridge Drive, Gaithersburg MD 20878 (e-mail: KXM@CDRH.FDA.GOV)

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MATERIALS AND METHODS

Cleaning Microorganisms from Materials

Organisms. *Staphylococcus epidermidis* RP62A (ATCC 35984), an organism known to adhere to polystyrene and medical implant materials,[5,6] *Candida albicans* (ATCC 14053), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and organisms from teeth scrapings were used in this study. All organisms were grown in trypticase soy broth (TSB) and used as stock cultures. The teeth scrapings (oral microorganisms) were a mix of aerobic or facultative organisms and were predominately Gram positive cocci.

A suspension of organisms, approximately 10^6 organisms/mL, was prepared in TSB and then 100 μL was placed into each well of the 96-well plate. The plates were incubated overnight at 37°C, and then washed 3 times with tap water, then finally treated with tap water or a cleaning agent. Some plates were treated with formalin or 70% alcohol before final cleaning.

Cleaning Agents. Tap water, diluted commercially available detergents with surfactants, detergents containing enzymes, enzymatic and nonenzymatic contact lens cleaning solutions, Triton X 100, a commercial mouthwash, peroxide based bleach, and bleach containing 5.25% sodium hypochlorite (NaOCl) were used. In addition, 10% neutral buffered formalin, benzyl ammonium chloride, and 70% ethanol were used. Commercially available high-level disinfectants: Cidex, Wavicide, and Cavicide were also used as directed on the labels.

Cleaning Protocol. The cleaning agent, 100 μL, was added to each well in 3 or 4 columns (24–32 wells) of the plate. Water was used in 3 or 4 columns as control. The plates were left to sit at room temperature for various time periods, washed with water again, and then assessed for efficacy of cleaning. There was no agitation or scrubbing of the wells, because this was a static test.

Assessment of Removal of Bacterial Biofilm. Neutral buffered formalin (10%) was added to the wells after cleaning to fix the remaining organisms in place, and the wells were then stained with crystal violet (1/10 dilution of Gram stain crystal violet).[4,5] The optical density, at 540 nm, of the stained adherent cells in the 96 wells was determined using a plate reader (Molecular Devices). A lower optical density indicates fewer organisms on the plate, hence more effective cleaning. The mean and standard deviation of the optical density was used for assessment.

Assessment of Sensitivity of the Dye Technique. Attempts were made to determine the number of organisms required to give an optical density greater than background. Several attempts were made, and the most successful technique was a culture technique. The organisms were incubated in the 96-well plates for various time intervals to allow them to adhere to the wells. The wells were then rinsed with sterile saline. Some of the wells were swabbed with a sterile cotton swab to detach the organisms. This swab was agitated in saline to remove organisms, and the number of organisms in the saline was determined by plate count on TSA. The 96-well plate was then stained in the usual manner.

Cleaning Protein from Materials

Calf serum was diluted 1/10 in phosphate buffered saline (PBS) and 100 μL was added to each well of the 96-well plates. A set of four plates was used in each experiment and incubated at room temperature for 24 h. One plate was cleaned immediately. The second plate was fixed in 70% ethanol, the third plate was fixed with formalin, and the fourth plate was allowed to dry. All four plates were then cleaned.

Albumin (ovalbumin and bovine serum albumin) was dissolved at 1 gm/mL in PBS and added to four 96-well plates at 100 μL per well. All four plates were incubated at room temperature for 24 h and then processed as described above for the plates treated with calf serum.

Bovine fibrin was purchased from Sigma and dissolved starting at 3 gm/30 mL of PBS. The protein did not completely dissolve, so the saturated supernatant solution was added at 100 μL per well to each of four 96-well plates. Similarly, bovine fibrinogen was purchased from Sigma and reconstituted to 3gms/30 mL of PBS. This formed a viscous solution and was added to four 96-well plates at 100 μL per well. The plates were all incubated for 24 h at room temperature, inverted and emptied, and then processed as described above for the plates treated with calf serum. Experiments with each protein were done 3 times.

Cells

To mimic cleaning of tissue from device materials, mammalian cell cultures were used. Murine macrophages (RAW 264.7) and murine fibroblasts L929 were grown in RPMI 1640 with 10% fetal calf serum in 96 well polystyrene plates at 37°C, with 5% CO_2. After the cells reached confluence, the medium was removed. As with the experiments with protein, for each cell type, one plate was washed immediately with water, a second received formalin, a third received 70% ethanol, and the fourth plate was left to dry at room temperature for 24 h. Experiments for each cell type were done 3 times.

Protein Stain. Naphthol Blue Black, Brilliant Blue R, and Bradford Reagent were purchased from Sigma, and all were effective at staining protein. However, Bradford Reagent proved to be the most sensitive (staining less than 1 μg of protein) and gave reproducible results. In addition, Bradford Reagent has the advantage that the solution changes color rather than staining the protein. This provides an advantage by allowing one to detect protein in small lumens, because the reagent can be inserted, incubated, and then removed; so one can measure quantitatively the amount of protein that was

on the surface, or in the lumen of any device with transparent or nontransparent materials.

Cleaning Procedure for Plates with Protein and Plates with Cells. Water was added to each well in the first 3 columns (24 wells) of the 96-well plate. Diluted bleach (0.525% NaOCl) was added to a second set of 3 columns, and detergent with enzyme was added to the wells in the third set of 3 columns. The last 3 columns were left empty and received only the final rinsing procedure. The plates were left at room temperature for 1 h, and then inverted to pour out the contents. The plates were rinsed by immersion in water and inverted to pour out the contents. This procedure was repeated 3 times. The plates were allowed to dry for 3–4 h before Bradford reagent was added. The plates were incubated at room temperature for 30 min and the optical density read at 600 nm with a spectrophotometer plate reader. The averages and standard deviations of the optical density readings for all 4 treatments (water, bleach, detergent, and rinse) were determined to evaluate cleaning efficacy. A minimum of 24 wells (3 columns) received the same treatment in each experiment. All experiments were repeated at least 3 times.

RESULTS

Organisms

The first study indicated differences between the organisms and their ability to adhere to the 96-well tissue culture polystyrene plates. *S. epidermidis*, *Candida albicans*, and the mouth organisms adhered well to the plates after 24 h of incubation at 37°C. *E. coli* did not adhere. *Pseudomonas* formed a heavy slime growth, which washed away easily with water leaving few residual organisms on the plate. The heavy slime of *Pseudomonas* was difficult to work with in the small wells of the 96-well format, so 24-well plates were used instead. Even in the larger wells, the slime adhered more to itself than to the polystyrene and the 24-well plate format could not be used in the plate reader. Thus, the data reported in this study are from mouth organisms, *Candida*, and *S. epidermidis*, which represent the worst cases for adherence.

Detergents

The detergents with surfactants cleaned only as effectively as water. The enzymatic contact lens cleaner did not clean very effectively. The mouthwash was good at removing the *S. epidermidis* and *Candida*, but not the mouth organisms. However, the detergents with enzymes removed all of the organisms very effectively.

Additional Studies Using Detergents with Enzymes. Many surgical instruments and devices are placed into chemical solutions containing aldehydes such as formalin or glutaraldehyde in the operating room and then are taken to central services for cleaning. There has been a general impression that disinfectants such as formalin cause the organisms to stick to surfaces and make cleaning more difficult. This was confirmed in this study. Wells containing organisms that had been fixed first with formalin and then washed had higher optical density readings, indicating less removal of organisms, than those wells that were washed in water only. Attempts to remove cells fixed with formalin with detergents containing enzymes were unsuccessful. Benzyl ammonium chloride, 70% ethanol, and liquid disinfectants behaved similarly to formalin.

Bleach

Hydrogen peroxide-based bleaches did not clean effectively. However diluted 5.25% sodium hypochlorite (NaOCl) cleaned very effectively. The recommendations on the bleach bottle to disinfect surfaces are equivalent to a 1/10 dilution. Therefore, studies were done with different dilutions of NaOCl in water. Dilutions from 1/10 to 1/50 were effective in removing the organisms. The 1/100 dilution was not effective.

The contact time with the biofilm was also studied. Cleaning with the 1/10 dilution sodium hypochlorite bleach (0.525% NaOCl) was effective in the shortest time period used, which was 15 min at room temperature.

Additional Studies with Bleach. NaOCl based bleach was then demonstrated to clean the wells under all conditions: it cleaned wells with adhered bacteria that were allowed to dry, that had been fixed first in formalin or liquid disinfectants, and those that had been cleaned, effectively or ineffectively, with detergents.

Because blood is often a contaminant of the medical devices being cleaned for reuse, studies were done with *S. epidermidis* RP62A incubated in TSB with 10% sheep blood. The data obtained indicated that the organisms in TSB/blood did not adhere as much to the polystyrene compared to the bacteria that were incubated in TSB alone. A 10% NaOCl-based bleach for 1 h at room temperature cleaned the plates of *S.* epidermidis with and without sheep blood. Microscopic analysis did not reveal any blood cells left after this bleach treatment. Both the detergents containing enzymes and the 10% NaOCl bleach were effective in removing the blood. Analysis for residual protein using Bradford reagent, brilliant blue R, and naphthol blue black stains indicated that NaOCl bleach removed residual protein.

Representative Numerical Results

The data obtained in representative studies on cleaning *S. epidermidis* biofilm formed in 96-well polystyrene plates for 24 h are shown in Table I. The averages and standard deviations from 32 wells receiving the same treatment are given. These results were replicated several times for *S. epidermidis*, *Candida*, and oral organisms. NaOCl bleach removed the biofilm effectively in all the treatment parameters.

Sensitivity of the method. Incubation of *S. epidermidis* in the 96-well plate for 5 min was sufficient to allow detection

TABLE I. The Optical Density Results from the Use of Different Agents to Remove 24-h *S. epidermidis* Biofilm from 96-Well Polystyrene Plates[a]

A: Plates rinsed with water and then washed for 2 h

	Water	Mouthwash	Enzyme Detergent	NaOCl Bleach
Average	0.230	0.065	0.054	0.041
Std dev	0.166	0.008	0.012	0.005

B: Plates rinsed with water, fixed with formalin, and then washed for 2 h

	Water	Mouthwash	Enzyme Detergent	NaOCl Bleach
Average	0.273	0.288	0.203	0.045
Std dev	0.056	0.059	0.194	0.001

C: Plates rinsed with water, fixed with quaternary amine, and then washed for 2 h

	Water	Mouthwash	Enzyme Detergent	NaOCl Bleach
Average	0.234	0.237	0.154	0.047
Std dev	0.040	0.044	0.043	0.004

[a] Plates had different treatments before cleaning.

of adherent organisms. The optical density of stained cells was significantly greater than in stained wells receiving only TSB or only dye. Detachment of the organisms from the well with a cotton swab gave the subsequent optical density readings similar to that of the TSB control. The cotton swab that was used to remove the organisms was agitated in saline. Culture of the saline revealed about 10^3 organisms per well exposed to bacteria for 5 min. The optical density increased over the time intervals (Table II) indicating an increase in number of adhering organisms. After 8 h of incubation, approximately 10^8 organisms/well were cultured. Thus, the use of 10% NaOCl bleach reduces the number of organisms by at least 10^4–10^5. The optical density is reduced, is indistinguishable from control, and is significantly below the 10^3 cells/well that could be present in less than 5 min of contact with the wells.

TABLE II. The Optical Density Results from Different Times for Adherence of *S. epidermidis* to 96-Well Polystyrene Plates

Time	Average	Standard Deviation
Control	0.045	0.001
5 min	0.050	0.002
10 min	0.050	0.002
15 min	0.056	0.005
30 min	0.088	0.021
1 h	0.102	0.020
2 h	0.156	0.060
3 h	0.268	0.014
4 h	0.410	0.150
5 h	0.534	0.220
6 h	0.834	0.220
7 h	1.23	0.31
8 h	1.25	0.22
24 h	1.61	0.34
48 h	0.98	0.27

TABLE III. The Optical Density Results from the Use of Different Agents to Remove Serum Protein from 96-Well Polystyrene Plates[a]

A: Plates washed immediately for 1 hour

	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.19	0.19	0.18	0.15
Std dev	0.004	0.009	0.02	0.006

B: Plates fixed with 70% alcohol and then washed for 1 hour

	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.33	0.31	0.17	0.16
Std dev	0.03	0.02	0.01	0.007

C: Plates rinsed fixed with formalin, and then washed for 1 hour

	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.18	0.18	0.22	0.14
Std dev	0.012	0.01	0.03	0.02

D: Plates allowed to dry for 24 hours and then washed for 1 hour

	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.23	0.69	0.16	0.16
Std dev	0.04	0.14	0.005	0.01

[a] Control of Bradfords reagent alone was 0.174.
Plates had different treatments before cleaning.

Protein

When the plates coated with serum protein were washed immediately, the protein was easily removed even with water only. However, other treatments before washing resulted in difficulties in removing the adherent protein. The results from one typical experiment (Table III) indicate that alcohol fixation or drying the plate affected the protein so that only the detergent with enzyme or the 10% bleach removed the protein. Fixation in formalin had minimal effect on subsequent ability to clean.

The results with fibrin and fibrinogen were similar, and the results from one typical experiment are shown in Table IV. Fixing the fibrin or fibrinogen in alcohol, in formalin, in liquid disinfectants, or letting the plate dry made subsequent cleaning very difficult. The 10% NaOCl bleach did the best job of removing fibrin or fibrinogen.

Cell Cultures

Two different mammalian cell lines were grown. Results were similar for the fibroblast L929 and macrophage RAW 264.7 cell lines. The results from a typical experiment using macrophages are shown in Table V and again show that 10% bleach is effective at removing the cells from the culture dishes. The detergent with enzyme removes the cells when

TABLE IV. The Optical Density Results from the Use of Different Agents to Remove Bovine Fibrin from 96-Well Polystyrene Plates[a]

A: Plates fixed with 70% alcohol and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.86	0.70	0.21	0.19
Std dev	0.13	0.08	0.05	0.02
B: Plates rinsed fixed with formalin, and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.57	0.54	0.51	0.18
Std dev	0.05	0.07	0.05	0.01
C: Plates allowed to dry for 24 hours and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	1.09	1.01	0.20	0.21
Std dev	0.05	0.05	0.02	0.02

[a] Control of Bradfords reagent alone was 0.169.
Plates had different treatments before cleaning.

washed immediately or allowed to dry, but does not remove the cells when they were first fixed with alcohol, formalin, or liquid disinfectants.

The results of microscopic examination of the plates were consistent with the data obtained with the stain.

CONCLUSIONS

This study focused on microorganisms, protein, and mammalian cells adherent to polystyrene as a "worst case" model.

TABLE V. The Optical Density Results from the Use of Different Agents to Remove a Monolayer of RAW 264.7 Cells from 96-Well Polystyrene Plates[a]

A: Plates fixed with 70% alcohol and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.58	0.55	0.47	0.18
Std dev	0.06	0.13	0.15	0.004
B: Plates rinsed fixed with formalin, and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.50	0.49	0.41	0.19
Std dev	0.05	0.07	0.05	0.01
C: Plates allowed to dry for 24 hours and then washed for 1 hour				
	Water	Rinse	Enzyme Detergent	NaOCl Bleach
Average	0.46	0.68	0.18	0.18
Std dev	0.04	0.05	0.004	0.008

[a] Control of Bradford's reagent was 0.174.
Plates had different treatments before cleaning.

TABLE VI. Summary of the Efficacy of Some of the Procedures Tested to Remove Biological Material from Polystyrene Plates[a]

	Water	Mouthwash	Detergent with Enzymes	NaOCl Bleach
Micro-organisms				
Wet	−	+	+	+
Formalin treated	−	−	−	+
Amine treated	−	−	−	+
	Water	Rinse Only	Detergent with Enzymes	NaOCl Bleach
Serum Proteins				
Wet	+	+	+	+
Dry	−	−	+	+
Alcohol treated	−	−	+	+
Formalin treated	+	+	+	+
Cells				
Wet	+	+	+	+
Dry	−	−	+	+
Alcohol treated	−	−	−	+
Formalin treated	−	−	−	+

[a] A + means that cleaning was effective, returning the optical density to not significantly different from the dye blank control. A − means that the cleaning was not fully effective and did not return the optical density to the level of the blank control.

Although polystyrene is not a material used in medical devices, other than for *in vitro* diagnostics, it has been shown to be an excellent material for promoting adherence of cells for tissue culture techniques and bacteria for assessment of biofilm formation.[5, 6] Although cleaning of devices would often involve scrubbing and the use of an ultrasonic cleaner, this study was designed to examine cleaning when neither of these would be possible and is an additional component of the "worst case" model. This study documented that detergents containing enzymes are effective at cleaning contaminated surfaces, and detergents without enzymes are no better than water. Table VI presents a summary of the findings for cleaning efficacy based on paired student *t*-test analyses. The documentation that hypochlorite bleach is effective in cleaning provides the possibility of a level of safety for the personnel handling these devices. This bleach is an effective low-level disinfectant capable of killing most, but not all, pathogenic organisms. Thus, a soak of at least 15 min in 10% hypochlorite based bleach (0.525% NaOCl) kills most of the pathogenic organisms. In addition, its use does not impair further cleaning efforts. Hypochlorite based bleach also aids in the cleaning of those devices that are allowed to dry or are disinfected with formalin before cleaning.

Although formalin and commercially available high-level disinfectants kill additional organisms that bleach does not, they do not clean the device and, in fact, impair further cleaning. In addition, they may be toxic to humans and to the environment.

Bleach with sodium hypochlorite (5.25% NaOCl) is a readily available agent, well documented for use in the home

and health care facilities.[7,8] Its efficacy for killing or inactivating many pathogenic bacteria, fungi, viruses, and parasites is well documented, and it does not harm the usual hospital environment when used appropriately.[7] However, its effect on various materials, such as plastics, metals, and fiber optics, used in medical devices needs to be assessed before it can be recommended for widespread use in multicomponent reusable devices being reprocessed several times.

The main message is: Do not let used medical devices that have been in contact with microorganisms, protein, or mammalian cells dry before cleaning, and clean devices thoroughly before disinfecting or sterilizing them.

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