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Major article

In vitro evaluation of cleaning efficacy of detergents recommended for use on dental instruments

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Background: The cleaning stage of instrument decontamination processes is a critical control point, and removal of protein deposits is used as a marker of cleaning efficacy. An important factor is the choice of cleaning solution especially in the absence of any defined standards for detergent effectiveness.
Methods: Following method validation, stainless steel tokens were inoculated with reconstituted citrated blood and added to a 24-multiwell plate and immersed in different cleaning solutions for 5 minutes, agitated at 25 (20°) tilts/min at 22°C and at the manufacturers' recommended temperatures. Desorbed protein was measured using the bicinchoninic acid assay.
Results: From a starting concentration with a median of 3,700 μg of blood protein of all solutions tested, alkaline detergent (Haemo-sol) removed the largest proportion of protein (median, 2,070 μg), and surgical handwash removed the least protein (median, 0 μg). Reverse osmosis water demonstrated useful blood-removing properties with a median of 1,421 μg.
Conclusion: The cleaning system we utilized is a simple, inexpensive method to compare the cleaning efficacies of detergents and may be used as a first stage in benchmarking cleaning efficacy of detergents. Not all solutions used in cleaning dental instruments are efficacious at removal of blood.

The cleaning of reusable medical devices is a critical control point in the decontamination cycle. The effectiveness of cleaning is important from a number of different perspectives such as ensuring accessibility of device surfaces to microbial inactivation processes,[1,2] device function,[3,4] freedom from harmful residues,[5,6] and compliance with various regulatory requirements.[7] Within many health care facilities, manual washing of surgical instruments prior to sterilization is frequently undertaken, especially in primary care settings. The effectiveness of manual cleaning has been questioned by a number of workers,[8,9] and observational studies have demonstrated a wide variation in policies and procedures used.[10] In particular, there is a wide variation in the type of cleaning solutions used, ranging from tap water only to surgical hand scrub, supermarket cleaning agents, and neutral detergents.[10]

The parameters that affect cleaning efficacy are summarized in Sinners circle, and these include the temperature of the cleaning solution/environment, the cleaning chemistries used, the duration of exposure to the cleaning process, and the amount of energy (mechanical, physical or ultrasonic) that is used during the cleaning stage.[11] In addition, it is important to consider the quality of the water used during the cleaning cycle, with dissolved solids and the hardness of the water having a detrimental effect on cleaning efficacy.[12] Optimizing the cleaning process by harmonizing the interactions of these variables can be challenging.[11] Whereas most variables can only increase or decrease, changing the cleaning solutions and the water quality may have multiple and subtle effects on the outcome of the cleaning process. Cleaning solutions have complex formulations and usually have several different active compounds[13] making comparisons between products difficult. Concerns have been raised over the effect of certain cleaning solutions on contaminant removal, with some studies demonstrating the fixing of blood and protein to surfaces.[14,15] Interestingly, there is no current standard or guideline, to the best of our knowledge, for determining the minimum standard for cleaning efficacy of chemicals used during the cleaning process.

For automated washer disinfector cleaning validation, the International standard BS- ISO/TS−15883-5: 2005[16] provides details of test soils to assess the efficacy of the washing processes. The test soil applied is dependent on the instruments or materials to be reprocessed and varies from defibrinated horse blood to complex formulations containing flour, blood, and bacteria. The efficacy of the cleaning process is measured using of 1 of 3 protein assays after

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Table 1
Properties of sampled detergents including manufacturers instructions

Cleaning solution (Study identifier)	Suitable cleaning systems	Manufacturers' instructions		
		Recommended dosage per liter H_2O	Recommended temperature	Measured pH
Alkaline 1	Manual Ultrasonic	10 g	"Cold, warm, or hot"	9.5
Enzymatic 1	Manual Ultrasonic AWD	4.25 mL	"Warm"	7.1
Alkaline 2	Manual Ultrasonic	2 g	"Warm, 50°C"	10.5
Handwash	N/A	N/A	N/A	5.0
Alkaline 3	N/A	28 g	"50°C" ("20°C-70°C acceptable")	11.0
Enzymatic 2	Manual Ultrasonic AWD	2 mL	"38°C"	7.2
Enzymatic 3	Ultrasonic	3.1 mL	N/A	5.5
0.1 mol/L NaOH	N/A	N/A	N/A	9.3
HP cleaning solution	N/A	100%	Room temperature	5.6

AWD, automated washer disinfector.

desorption of residual protein from the test pieces or instruments BS EN ISO 15883-1: 2009.[17] Previous work into cleaning efficacy has utilised either the in vivo process or involved the development of models based on various cleaning parameters. For some complex models, especially those utilizing pressurized jets of water, it is difficult to determine the precise effect the cleaning solution is having on cleaning efficacy.[8] A simpler model to investigate the effect of cleaning chemistries can help inform optimization of the cleaning process and procurement decision making.

The widespread use of a disparate group of chemicals used in the manual cleaning of dental instruments[10] and plethora of chemicals available to purchase prompted us to investigate assembling a simple in vitro test for benchmarking of cleaning efficacy by a range of cleaning chemistries focusing on primary care manual cleaning applications.

METHODS

Chemicals and substrates

All chemicals were obtained from Sigma Aldrich (Poole, Dorset, UK) unless otherwise stated. All blood products were acquired from E & O Laboratories (Bonnybridge, UK). Reverse osmosis water (RO H_2O) was obtained from a Purelab Prima DV 34 unit (Elga Water, Glasgow, Scotland). Stainless steel sections (SSSs) made of 304 medical grade measuring 10 mm by 10 mm and thickness of 1 mm were a gift from Dr K. Smith (University of Glasgow). Detergents and cleaning solutions used in this study were acquired from the manufacturer. Each cleaning solution was given a study identifier name based on the cleaning solution properties as follows; Alconox (Alkaline 1), Haemo-sol (Alkaline 2), Rapidex (Alkaline 3), Endozime AW+ (Enzymatic 1), Rapizyme (Enzymatic 2), Sonozyme (Enzymatic 3), HibiScrub (Handwash), and W&H handpiece lubrication solution (HP cleaning solution) (Table 1). Mains supply water was obtained from the Glasgow Dental Hospital supply on the day of the experiment. The pH, conductivity, salinity, and total dissolved solids of each water sample and, where appropriate, detergents were determined using a PCSTestr 35 (Eutech Instruments, Nijkerk, Holland) (Table 2).

Preparation of stainless steel surfaces

Prior to each experiment, the SSS were cleaned by immersing in 0.1 mol/L sodium hydroxide (NaOH), pH 9.2, and boiled for 10 minutes. The discs were then rinsed with methanol (BDH laboratories, Leicester, UK) and dried in a laminar flow cabinet for 1 hour.

Table 2
Properties of H_2O used in the study

H_2O source	Measured pH	Conductivity (μS)	Salinity (ppm)	Total dissolved solids (ppm)
Tap	6.41	63.7	35.6	46.2
Reverse osmosis	5.49	2.3	12.5	3.6

Test soil and inoculation of discs

The test soil used was the Swedish test soil detailed by the ISO/TS 15883-5: 2005.[16] Briefly, test soil was made by adding 1 mL of 0.1 mol/L calcium chloride ($CaCl_2$) (Difco, Oxford, UK) to 9 mL of citrated horse blood. The SSSs were inoculated with 30 μL of solution of recalcified horse blood, and negative controls comprised SSSs with 30 μL of 0.1 mol/L $CaCl_2$. Each inoculated SSS was air-dried for 16 hours at ambient room temperature. Each SSS was then inserted into a Costar 24-well plate. For each experimental run, controls comprised a protein assay for 30 μL of citrated blood diluted in 1 mL of RO H_2O. The proportion removed was expressed as the quantity of protein removed by the experimental wash compared with the protein detected in the control. The 24-well plates, containing inoculated SSSs, were then placed on the center of a PMR-30 rocking platform (Grant Instruments, Cambridge, UK), which tilts at 20° from the horizontal. Each well containing a SSS was subsequently challenged with 1 mL of the appropriate cleaning solution. The experiment consisted of 3 × SSS, and the experiment repeated in triplicate.

Optimizing cleaning parameters

The effect of 3 cleaning parameters on blood removal was assessed: (1) cleaning time, (2) temperature of solutions, and (3) agitation speed of the platform. For the effect of agitation time on blood removal, the rocking platform was set to a speed of 25 (20°) tilts/min, and 100 μL samples were taken at 1, 5, 10, 15, and 20 minutes. To investigate the effect of RO H_2O temperature on blood removal, the tilting platform was set to 25 (20°) tilts/min, and each well containing a disc was inoculated with 1 mL of RO H_2O at the temperatures of 22°C, 38°C, and 50°C based on the range of manufacturers recommended cleaning solution temperatures