Effect of hand hygiene and glove use on cleanliness of reusable surgical instruments


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SUMMARY

Background: During functionality testing and packaging of reusable surgical instruments (RSI) for sterilization, instruments are frequently touched. There is a lack of standards relating to hand hygiene frequency and use of gloves in the sterilizing service unit packing area.

Aim: To determine the effect of hand hygiene and glove use on maintenance of RSI cleanliness.

Methods: Following manual and automated cleaning, Halsted-mosquito forceps were assessed for adenosine triphosphate (ATP), protein and microbial contamination after handling with gloved and ungloved but washed hands using an ATP surface swab test, bicinchoninic acid assay, and standard culture plate/broth, respectively. Gram's stain was used to classify the isolates. RSI contamination was assessed immediately following and 1, 2, and 4 h after washing hands.

Findings: Packing instruments with hands that had been unwashed for 2 or 4 h resulted in a significant increase in contaminating ATP when compared with all other treatment groups (P < 0.05). There was a significant correlation between the time since washing hands, the amount of ATP (r = 0.93; P ≤ 0.001), and the microbial load (r = 0.83; P ≤ 0.001) contaminating the forceps, where the longer the time the hands remained unwashed the higher the contamination. Significantly more contaminating protein was found on forceps handled with ungloved hands that had not been washed for 2 or 4 h (P < 0.001).

Conclusion: Critical RSI inspection, assembling, lubricating and packing should be performed using either gloves or within 1 h of washing hands.

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Introduction

Processing of reusable surgical instruments (RSI) is crucial for ensuring that infectious agents are not transmitted by contaminated instruments during surgery. This process is complex and encompasses a chain of interdependent steps from the point-of-use to storage and subsequent distribution.
Cleaning is considered the most important step, for it removes organic and inorganic matter, thus reducing the initial microbial load on instruments for subsequent disinfection or sterilization, and for their safe handling by sterilizing services unit (SSU) personnel during packing [1,2].

Sterilization of critical RSI is required; following cleaning, instruments must be packed in a suitable sterile system barrier [1,2]. In the packing area, cleaned instruments are inspected, assembled (if applicable), lubricated (if applicable), and finally packed. Thus, they are handled multiple times by SSU personnel. Standard hand hygiene requirements in the SSU clean area include: on entering and leaving the unit, before putting on gloves and after removing them, and at the beginning and end of the work shift [3]. These standard hand hygiene moments have been shown to be insufficient to ensure cleanliness of instruments; therefore, ‘additional’ moments of hand hygiene have been suggested for the packing area [4]. These include: before donning protective clothing; before receiving and folding surgical drapes, etc.; before assembling and loading boxes and trays; before preparing Bowie and Dick test; after incubating and after disposal of biological indicator; after administrative activities such as use of telephone, computer and minute books; and after disinfecting benches. However, a maximum standard time interval for hand hygiene has not been suggested. Moreover, similar to other healthcare areas, failure to perform hand hygiene in the SSU is a frequent event [4].

The use of personal protective equipment such as scrubs, head wear, mask and gloves is recommended in the packing area by some standards, but this is by no means universal [1,2,5–7]. Furthermore, glove use recommendations are not based on scientific evidence.

In view of the lack of unanimous worldwide agreement on whether gloves should or should not be used, and the lack of evidence for the frequency of hand hygiene when packing instruments prior to sterilization, we aimed to investigate these factors by determining instrument contamination during handling in the packing area. Adenosine triphosphate (ATP), protein and bacterial contamination of RSI were assessed following packaging.

Methods

Patient-ready clean and sterile Halsted-mosquito forceps (AS Medizintecnik GmbH, Tuttinglen, Germany) were subjected to manual cleaning in Prolystica™ 2 x concentrate enzymatic presoak and cleaner (Steris, Mentor, OH, USA), rinsed in 0.2 μm filtered water and then subjected to automatic cleaning in a Reliance Vision Single-Chamber Washer/Disinfector (Steris Corporation, Beauport, Quebec, Canada). The washer–disinfector cycle included two wash phases, the first using an enzymatic detergent and the second an alkaline detergent; these phases were followed by a thermal rinse and a final rinse using reverse osmosis water.

Contaminating ATP

ATP is an intracellular energy source present in all living cells including micro-organisms, and is thus a measure of organic soil. The amount of ATP contaminating items is quantified by measuring the amount of light produced through its reaction with luciferase, and is expressed in relative light units (RLU).

The amount of ATP present on forceps pre-handling (N = 45) was determined using the Clean-Trace™ Surface ATP Test Swab UXL100 (3M, Bracknell, UK); the test was performed using an aseptic technique and wearing sterile gloves. The swab was rubbed on the hinged and the serrated areas of the forceps while the forceps were in both the open and closed position to ensure that the whole area was sampled. ATP was read using the Clean-Trace™ NG Luminometer (3M) following the manufacturer’s instructions.

Each forceps was then subjected to one of the following handling treatments (N = 9 per test group) while being inspected and packed in a sterile barrier system (spunbonded–meltblown–spunbonded):

- Group 1: RSI handled with clean, non-sterile nitrile gloves;
- Group 2: RSI handled with ungloved hands immediately following hand hygiene (time zero);
- Group 3: RSI handled with ungloved hands 1 h after hands were washed;
- Group 4: RSI handled with ungloved hands 2 h after hands were washed;
- Group 5: RSI handled with ungloved hands 4 h after hands were washed.

In addition, a control group using RSI that were aseptically handled using sterile surgical gloves was included. Hand hygiene was performed according to recommended World Health Organization technique [1]. Immediately after packing the forceps, the amount of contaminating ATP was determined as described above. Pre- and post-ATP tests were matched for each forceps.

Microbial load

Following cleaning, forceps were aseptically inspected and packed while wearing sterile surgical gloves (unhandled control) or inspected and packed using ungloved hands that had been washed 1 h (group 2), 2 h (group 3), and 4 h (group 4) previously. Following unpacking, forceps were tested qualitatively and quantitatively for presence of bacterial contamination.

For the quantitative assessment nine artery forceps per test group were immediately immersed in 10 mL of phosphate-buffered saline (PBS) and sonicated (Soniclean™, JMR, Mount Kuring-Gai, NSW, Australia Australia) for 10 min to remove attached bacteria. Bacteria were concentrated by filtering through a 0.22 μm membrane (MS MCE membrane filter, Membrane Solutions, Kent, WA, USA). The membrane was placed on to horse blood agar (HBA) and incubated for up to 48 h at 37°C and colony-forming units (cfu) counted.

For the qualitative assessment nine artery forceps per test group were incubated in 10 mL tryptic soy broth (TSB), for up to 48 h at 37°C. A 10 μL aliquot of positive cultures was sub-cultured on HBA.

Colonies isolated on HBA were presumptively identified by using macroscopic features and Gram stain (Accustain™ Gram stain, Sigma–Aldrich, Inc., Saint Louis, MO, USA).

Residual protein

Following cleaning, forceps were aseptically inspected and packed while wearing sterile surgical gloves (unhandled
control) or inspected and packed using ungloved hands that had been washed 1 (group 2), 2 (group 3) and 4 h (group 4) previously \((N = 9\) per test group). Following unpacking, the amount of contaminating protein was determined using the bicinchoninic acid (BCA) protein assay (Pierce™; Thermo Fisher, Waltham, USA), according to the manufacturer’s instructions for use.

**Statistical analysis**

The statistical package SigmaPlot 13 was used for all statistical analysis. The data needed to be transformed to ensure normality. To test for differences in the amount of ATP contaminating unhandled and handled forceps, a paired \(t\)-test was used if normally distributed or a Wilcoxon signed-rank test was used if not normally distributed. A one-way analysis of variance repeated measures analysis of variance, using the Holm–Sidak method of all pairwise multiple comparisons, was used to test the relationship between the different treatments. A linear regression model was used to determine whether the number of contaminating bacteria or amount of contaminating ATP was related to the time since hand hygiene had been performed.

**Results**

**Contaminating ATP**

The mean amount of ATP contaminating forceps that were handled with sterile surgical gloves (unhandled control group) was 23.5 RLU (range: 9–68). Irrespective of whether non-sterile gloves or bare hands were used to handle forceps, significantly more ATP was found to contaminate handled forceps post packaging when compared with the amount of ATP contaminating the unhandled control forceps \((P < 0.05)\) (Figure 1).

The amount of contaminating ATP was similar for instruments packed using non-sterile gloved hands or if packed using ungloved hands immediately following their washing \((P > 0.05)\). Packing of instruments if hands had been unwashed for \(\geq 1\) h resulted in a significant increase in amount of contaminating ATP \((P < 0.05)\). Packing instruments with hands that had been unwashed for 2 or 4 h resulted in a significant increase in contaminating ATP when compared with all other treatment groups \((P < 0.05)\) (Figure 2). There was a significant correlation between the time since washing the hands and the amount of ATP \((r = 0.93; P \leq 0.001)\) contaminating the forceps, where the longer the time the hands remained unwashed the higher the ATP contamination.

**Bacterial contamination**

All bacterial cultures of unhandled forceps were negative. All handled forceps incubated in TSB (qualitative assessment) grew bacteria and were excluded from further analysis. Mean microbial loads of \(1.1 \times 10^1\), \(1.9 \times 10^1\) and \(2.5 \times 10^1\) were found to be contaminating forceps handled using ungloved hands after 1, 2 and 4 h since washing. There was a significant correlation between the time since washing hands and the resulting microbial load (cfu) contaminating the forceps \((r = 0.83; P \leq 0.001)\) where the longer the time since washing hands the higher the contamination (Figure 3). Bacteria were recovered from all artery forceps that had been handled with ungloved hands. Non-haemolytic, Gram-positive cocci with colony morphologies resembling coagulase-negative staphylococci were the most frequently isolated organisms. Gram-negative and Gram-positive bacilli were also isolated.

**Residual protein**

No protein was detected on unhandled forceps. Handling of forceps with ungloved hands resulted in detection of contaminating protein. Significantly more contaminating protein was found on forceps handled with ungloved hands that had not been washed for 2 h (mean: 1750 μg; range: 1258–2466) or 4 h (mean: 1973 μg; range: 555–3551) \((P < 0.001)\) (Figure 4).

![Figure 1. Contaminating adenosine triphosphate (RLU) present on clean, unhandled (control group: black bar) Halsted-mosquito forceps and following packing with gloved hands (group 1); ungloved hands immediately following washing: time 0 (group 2); 1 h after handwashing (group 3); 2 h after handwashing (group 4); and 4 h after handwashing (group 5). *\(P < 0.05\).](image-url)
Discussion

In the packing area of SSU, personnel usually perform the same activity or task for extended periods of time and an observational study showed that compliance with hand hygiene is suboptimal [4]. In this study, we showed that the method of handling clean instruments during inspection, assembly, and packaging determines the degree to which the instruments become contaminated. Even packing instruments using non-sterile nitrile gloves and recently washed bare hands increased the amount of contaminating ATP (180% and 266%, respectively) when compared with aseptic handling. However, packing instruments with hands that had not been washed for 1 h resulted in ATP contamination levels above the suggested benchmark of 100 RLU [8,9]. Increasing the time during which hands remained unwashed to 2 h increased ATP contamination 5.6-fold \((P < 0.05)\). Further delay in handwashing increased ATP contamination non-significantly but significantly increased microbial contamination \((P < 0.05)\).

Instruments removed from washer-disinfectors are generally microbiology negative, but will become contaminated with bacteria if bare hands are used. As with ATP, there was a significant correlation between time since handwashing and increased microbial contamination. Gram-negative bacilli were among the organisms detected in this study and present a risk of endotoxin contamination.

Protein contamination of patient-ready RSI is also a concern \([10–12]\). Residual protein on properly processed instruments should be very low: the most recent proposed benchmark is 5 \(\mu\)g (BCA assay sensitivity) protein residue per side of instrument

![Figure 2. Contaminating adenosine triphosphate (relative light units: RLU) present on clean, unhandled (control group) Halsted-mosquito forceps and following packing with gloved hands (group 1); ungloved hands immediately following washing: time 0 (group 2); 1 h after handwashing (group 3); 2 h after handwashing (group 4); and 4 h after handwashing (group 5). *Significantly less than all other handled groups \((P < 0.05)\); groups 1 and 2: significantly less than groups 3, 4, and 5; group 3: significantly greater than groups 1 and 2, and significantly less than groups 4 and 5 \((P < 0.05)\).](#)

![Figure 3. Microbial load (cfu: colony-forming units) contaminating Halsted-mosquito forceps following packing with ungloved hands washed after 1 h (group 3), 2 h (group 4), and 4 h (group 5) compared with unhandled control forceps. *\(P < 0.05\).](#)

![Figure 4. Protein contaminating Halsted-mosquito forceps pre-packing (unhandled control) and following packing with ungloved hands washed 1 h (group 3), 2 h (group 4), and 4 h (group 5) previously. *Significantly greater than unhandled control forceps \((P < 0.001)\).](#)
Handling instruments with hands that had not been washed for an hour resulted in an average of 577 μg protein per instrument which is more than 50-fold higher than recommended. The amount of protein remaining on instruments increased rapidly as the time since washing hands increased: by 2 h protein contamination was three-fold higher. However, what is more concerning is that the large amount of protein from workers’ hands, added to instruments during packaging, could help protect prion protein from decontamination. Both prion protein and endotoxins are thermostable and remain unaffected by conventional sterilization processes [2].

Pires et al. suggested that there should be a set time interval for handwashing in the clean areas of SSU, including the packing area, and that the interval should be based on the time that transitory microbiota regrow following washing hands [3,4]. Additionally, SSU environmental surfaces may harbour infectious agents, and pose a hand and RSI contamination risk. Our results show that microbial load on instruments almost doubled, that protein contamination tripled, and that ATP readings were 5.6-fold higher on instruments packed 2 h after handwashing when compared with contamination on instruments packed 1 h after handwashing. This suggests that, to minimize instrument contamination, ungloved hands should be washed at least hourly. However, given the likely low compliance with hand hygiene, a change in SSU worker attitude is needed, which will require establishment of education and monitoring programmes, if gloves are not worn [3].

In conclusion, cleaning aims to remove the bioburden from instruments, as high bioburden compromise sterilization [2]. In order to avoid contamination of instruments following cleaning, gloves should be worn or hands should be washed each hour in the SSU packing area.

Conflict of interest statement
None declared.

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