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Concise Communication

Does blood on "dirty" instruments interfere with the effectiveness of sterilization technologies?

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Abstract

We evaluated the robustness of sterilization technologies when spores and bacteria were placed on "dirty" instruments and overlaid with blood. The results illustrate that steam sterilization is the most effective sterilization technology with the largest margin of safety, followed by ethylene oxide and hydrogen peroxide gas plasma.

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Surgical instruments that enter sterile tissue should be sterile because microbial contamination could result in disease transmission.[1] Despite careful surgical instrument reprocessing (cleaning and sterilization), surgeons and other healthcare personnel (HCP) describe cases in which surgical instruments have been contaminated with organic material (eg, blood). Although most of these cases are observed before the instrument reaches the patient, in some cases the contaminated instrument contaminates the sterile field, or rarely, the patient. Only a few studies have evaluated the robustness of sterilization technologies when spores and bacteria are placed on instruments in the presence of protein, salt, or lubricating oils and the effectiveness of the sterilization technologies was assessed.[2–4] This study is the first to evaluate the robustness of sterilization technologies when spores and bacteria were placed on "dirty" (uncleaned) instruments and overlaid with blood.

Methods

For the test organisms, we used methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa*, *Mycobacterium terrae*, *Geobacillus stearothermophilus* spores, and *Bacillus atrophaeus* spores. The spores used were most resistant to the sterilization process tested (eg, *Geobacillus stearothermophilus* for steam and hydrogen peroxide gas plasma [HPGP] and *B. atrophaeus* for ethylene oxide [ETO]). In brief, our study method follows. "Dirty" surgical instruments (uncleaned) were inoculated with the 1.5×10^5 to 4.1×10^7 test organisms with or without blood, were subjected to a sterilization process, and then the instruments were cultured. The test organisms (except *M. terrae*) were grown in trypticase soy broth (TSB) with 5% fetal calf serum (FCS). For *M. terrae*, a frozen suspension was thawed and placed in TSB with 5% FCS. The "sandwich" technique used, consisting of 3 layers: a 'dirty" (uncleaned) instrument (ie, scissors, hemostats, retractors, rasps, forceps, needle holders, clamps); the inoculum of spores or vegetative bacteria in the middle; and the dried microbial inoculum was overlaid with human blood. Once the inoculum was dried for 1 hour in a biological safety cabinet, the instruments were placed in a peel pouch and sterilized by steam sterilization (cycle of 4 minutes at 270°C; Steris Amsco, Mentor, OH), ETO (100% ETO, Steris Amsco), or hydrogen peroxide gas plasma (Sterrad 100S, Advanced Sterilization Products, Irvine, CA). After sterilization, each inoculated instrument was aseptically placed in an immersion bath that contained 200 mL or 400 mL TSB, allowed to shake for 1 hour on a clinical rotator and then the TSB was filtered (Savur analytical filter, Fisher Scientific, Waltham, MA), incubated on appropriate media (sheep blood agar or Middlebrook 7H11 agar [*M. terrae*]), at appropriate temperature (ie, 37°C or 55°C) and incubation times (ie, 48 hours or 21 days [*M. terrae*]), and the microbial growth was recorded. The failure rate was calculated as follows: number of failures per number of tests ×100. These experiments are not representative of practice or manufacturer's recommendations because cleaning must always precede sterilization.[1]

Results

Steam sterilization inactivated all the *G. stearothermophilus* spores, *M. terrae,* MRSA, and VRE when inoculated onto "dirty" instruments (uncleaned) in the presence and/or absence of blood (Table 1). ETO failed to inactivate all *B. atrophaeus* spores when inoculated onto "dirty" instruments (60% failure) and "dirty" instruments with blood (90% failure). ETO did kill the vegetative bacteria (ie, MRSA, VRE) and *G. stearothermophilus* spores under the same test conditions (ie, "dirty" instruments with and without blood). The failure rates for HPGP were 60% for *G. stearothermophilus* spores, 90% for VRE, and 40% for MRSA when blood overlaid the inocula on "dirty" instruments. When "dirty" instruments were inoculated in the absence of blood, the

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Table 1. Effectiveness of the Microbicidal Activity of Sterilization Technologies in the Presence of Blood on "Dirty" Instruments[a]

Test Organism	Method of Sterilization	Instruments "Dirty" (Uncleaned) With or Without Blood[b]	Instrument Quantitation (Mean)	No. of Positives/ No. of Runs (% Positive)
Geobacillus stearothermophilus (spores)	Steam Sterilization	Dirty	~ 1.56×10^5	0/10 (0)
		Dirty with blood (spores mixed with blood not sandwich[b])	~1.99×10^5	0/12 (0)
	ETO	Dirty	~1.53×10^5	0/10 (0)
		Dirty with blood	~2.35×10^5	0/11 (0)
	HPGP	Dirty	~1.58×10^5	5/10 (50)
		Dirty with blood	~2.35×10^5	9/15 (60)
Mycobacterium terrae	Steam Sterilization	Dirty	~4.25×10^6	0/10 (0)
P. aeruginosa	HPGP	Dirty	~1.81×106	3/15 (20)
Bacillus atrophaeus (spores)	ETO	Dirty	~ 2.30×10^7	6/10 (60)
		Dirty with blood	~4.08×10^7	9/10 (90)
MRSA	ETO	Dirty	~2.62×10^6	0/10 (0)
		Dirty with blood	~1.72×10^6	0/10 (0)
	HPGP	Dirty	~1.10×10^6	4/10 (40)
		Dirty with blood	~1.27×10^6	4/10 (40)
	Steam sterilization	Dirty	2.56×10^6	0/10 (0)
		Dirty with blood	5.20×10^5	0/10 (0)
VRE	ETO	Dirty	~2.27×10^6	0/10 (0)
		Dirty with blood	~3.59×10^6	0/10 (0)
	HPGP	Dirty	~2.63×10^6	3/10 (30)
		Dirty with blood	~2.34×10^6	9/10 (90)
	Steam sterilization	Dirty	1.90×10^6	0/10 (0)
		Dirty with blood	2.72×10^5	0/10 (0)

Note. ETO, ethylene oxide; HPGP, hydrogen peroxide gas plasma; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*.
[a]Study conditions not representative of practice or manufacturer's recommendations.
[b]Sandwich consists of "dirty" or uncleaned instrument, then an inoculum of spores or vegetative bacteria, and lastly overlaid with blood after inoculum dry. One G. stearothermophilus experiment was done with the spores mixed with the inoculum and then placed on the dirty instrument.

failure rates for HPGP were 50% for *G. stearothermophilus* spores, 30% for VRE, 40% for MRSA and 20% for *P. aeruginosa*.

Discussion

Sterilization technologies are essential for instrument reprocessing in healthcare facilities. Cleaning, or the removal of visible soil (eg, organic and inorganic material) and microbial contaminants from objects and surfaces, precedes sterilization. Cleaning should consistently and reliably remove and/or reduce the organic and inorganic materials before sterilization to not interfere with the effectiveness of sterilization and to ensure a sterility assurance level of 10^{-6}.[1] The criticality of cleaning was reconfirmed by Alfa et al[2] in a classic study that evaluated various low-temperature sterilization technologies and showed sterilization failure in the presence of salt, serum, and lumen test units. Given that organic material and salts are known to influence the sterilization capacity of low-temperature sterilization technologies,[1–5] we evaluated the impact of inadequate cleaning and blood on the efficacy of the sterilization technologies used in the United States.

The frequency of instrument contamination in the operating room is poorly described in the literature. When instruments are contaminated with organic material from a prior procedure, healthcare personnel refer to that as "bioburden." One study found contamination problems in the operating room in ~1% of surgical instruments, and ~50% of the contamination type reported by the operating room staff was bioburden (ie, organic material such as blood or tissue), which was categorized differently than foreign bodies in the tray (eg, hair or object).[6] Rarely do these instruments with bioburden touch the patient; however, there is a potential for contaminating the surgical field. In this study, we assessed this instrument contamination issue and the cliché, "If it's not clean, it's not sterile."

Our results showed that steam sterilization is the most robust (ie, has the greatest ability to withstand and overcome adverse conditions or rigorous testing) sterilization process. It was effective even when instruments were not cleaned ("dirty" instruments) and the test organisms (*G. stearothermophilus* spores, *M. terrae*) were mixed or overlaid with blood. Low-temperature sterilization technologies tested failed to inactivate the test spores (ie, *B. atrophaeus* for ETO and *G. stearothermophilus* for HPGP) on "dirty" instruments with or without blood. ETO did kill the vegetative test bacteria (ie, MRSA and VRE), but HPGP did not kill *P. aeruginosa*, MRSA, or VRE on "dirty" instruments with or without blood.

These results demonstrated that some sterilization technologies were more "forgiving" or safe when cleaning is incomplete. The robustness of some sterilization technology, such as steam sterilization, makes it exceedingly unlikely that a steam-sterilized

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instrument will be the source of infection. These findings should assist healthcare personnel in assessing the risk of infection to patients when potentially contaminated surgical instruments enter the sterile field or are unintentionally used on patients during surgery.

The findings regarding the robustness of steam sterilization should not be used to suggest that cleaning is unimportant for steam sterilization. In contrast, the data demonstrate how important cleaning is prior to sterilization because salt and organic matter left on instruments can interfere with low-temperature sterilization. These results, and those of other investigations,[2–5] highlight the importance of real-time monitoring methods prior to sterilization that are reliable, validated, and assess the effectiveness of cleaning that is predictive of microbial contamination, infection risks, and/or a sterility assurance level of 10^{-6}.[7] Current assessment tools (eg, visual and adenosine triphosphate [ATP]) are not predictive of microbial contamination or infection risks. Investigators have demonstrated that visual assessment and ATP lack the sensitivity required to ensure effective decontamination.[8]

Our research was limited by the fact that we did not assess other factors than might affect sterilization, such as microbial contamination in lumened instruments, or transfer and storage conditions, which could enhance microbial growth or biofilm formation.

These results illustrate that steam sterilization is the most effective with the largest margin of safety, followed by ETO and HPGP. The data demonstrate how important cleaning is prior to sterilization because salt, blood, oils, and organic matter left on instruments can interfere with low-temperature sterilization.[1–5] The issue of "how clean is clean enough" remains unresolved but various cleaning markers (eg, protein ≤6.4 μg/cm^2) of when to consider a device clean can be used by manufacturers when preparing reprocessing instructions for users.[9,10] However, these cleaning markers must be available in real-time to instrument reprocessing technicians.

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Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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