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Optimizing the Glass Bead Sterilization Protocol Focusing on Removal of Organic and Bacterial Intraoperative Contamination

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Validated glass bead sterilization protocols to effectively sterilize rodent surgical instruments after bacterial exposure (for example, cecal contamination) are lacking. To refine current approaches, we added either a multienzyme detergent, neutral pH detergent, or chlorhexidine scrub step before glass bead sterilization of forceps or needle drivers exposed to cecal contents. We exposed sets of forceps and needle drivers to cecal contents, which were then air dried for 3 min. Immediately after, the instruments were wiped several times with a clean, dry paper towel. The contaminated tips were soaked in either a multienzyme or neutral pH detergent (t = 5 min), chlorhexidine scrub (t = 2 min), or no pretreatment solution. To further increase debris removal, instruments (from all groups) were brushed using a clean toothbrush. The nonpretreatment instruments were briefly soaked in saline before brushing. After being rinsed with sterile water, all instruments were exposed to a glass bead sterilizer for 60 s at 500 °F (260 °C). Sets were then swabbed for bacterial culturing. Swabs were plated onto either sheep blood agar (n = 23) or chocolate agar (n = 20) for aerobic culturing or *Brucella* agar (n = 20) for anaerobic culturing. A subset of instruments was sampled to determine organic material presence after treatment using an ATP luminometer (n = 21). Multiple agar types and bioluminescence were used to more deeply evaluate tool sterility and to differentiate the relative effectiveness of each protocol. From the saline group, only one pair of forceps yielded growth on *Brucella* agar, and 2 pairs yielded growth on chocolate agar. No other bacterial growth was observed. The use of a pretreatment agent also lowered overall organic contamination levels in needle drivers compared with using only saline. These results indicate that brushing instruments to mechanically remove debris from instruments is paramount to ensure sterility. However, a best practice would be to also use one of the pretreatment options used in this study.

Abbreviations and Acronyms: GBS, glass bead sterilizer; RLU, relative light units

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Introduction

Maintaining a strict aseptic technique is critical to reducing the likelihood of postsurgical infections in rodent surgeries. Numerous studies have examined different presurgical skin preparation methods and their effectiveness in reducing bacterial skin contaminant bacteria.[2,6,9,12] Maintaining the sterility of surgical instruments is also vital to preventing postsurgical infections. Previous work has revealed that even subclinical infections can impact research outcomes.[4,8] In experiments involving rodent surgeries, multiple surgeries are often carried out sequentially (for example, often referred to as "batch surgeries"). It is often impractical to perform surgery using a new set of autoclaved instruments for each animal within a batch.

In rodent batch surgeries, a glass bead sterilizer (GBS) is commonly used to sterilize surgical instruments between one surgery and the next. Recent interest has been in GBS protocols to best ensure the microbial load on surgical instruments is lowered as much as possible between animals. In one study,[17] various surgical instruments were submerged in bacterial solutions containing either *Escherichia coli* or *Staphylococcus aureus* to simulate intestinal and skin bacterial contamination, respectively. Wiping each instrument with an ethanol wipe and placing it individually in the GBS for 15 s resulted in the sterilization of only 82.5% of the instruments. No significant difference existed between the likelihood of eliminating either bacterial species tested.

Another study[10] examined the effectiveness of using a GBS in the face of skin and cecal contamination during mouse laparotomies. This study also investigated the maximum number of surgeries using interoperative glass bead sterilization that would yield sterile surgical instruments. After each surgery, the forceps and needle holders were cleaned of visible debris using sterile saline, placed in the GBS together (t = 60 s), and allowed to cool (t = 30 s). Most instruments used under aseptic conditions (control) and those mimicking skin contamination were considered sterile after this protocol. However, 25% of instruments exposed to cecal contamination were contaminated posttreatment. For instruments exposed to aseptic conditions or those that mimicked skin contamination, after 4 surgeries they only had an 80% chance of being sterile. Likely the 60-s period the instruments were in the GBS was sufficient for

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sterilization. However, particularly for the instruments exposed to cecal contamination, gross debris remained posttreatment, which could have served as a nidus for bacterial contamination.

Different solutions are available to aid in the removal of gross debris from surgical instruments after usage. First, multienzyme medical instrument cleaners rapidly dissolve fat, blood, mucus, and other proteinaceous materials encountered during surgical procedures.[13] Next, there are several neutral pH detergents commercially available to remove debris from surgical instruments after usage. Neutral pH detergents use chelating agents and naturally occurring proteins to remove contamination. They contain surfactants, and their nature as a detergent also provides lubrication, which makes manual removal of gross debris after soaking easier.[7] Finally, though not typically used as an instrument cleaning option, chlorhexidine scrub is a common antiseptic used to remove surface microbial contamination on the skin before surgery. The combined scrub-like nature for the removal of surface contamination and antimicrobial activity makes it a suitable candidate to enhance glass bead sterilization.

Furthermore, in testing the efficacy of glass bead sterilization, previous studies used only general-purpose bacterial media (such as sheep blood agar) to assess post-GBS bacterial growth.[10,15,17] While many of the potential bacterial contaminantspecies will grow on such media, many will not.[18] Therefore, general-purpose bacterial media may not be sufficient to effectively evaluate contamination. Chocolate agar is considered an enriched version of sheep blood agar where the erythrocytes have been lysed, releasing intracellular nutrients such as hemoglobin, hemin (X factor), and NAD+ (V factor) into the media.[3] These nutrients are used by many fastidious bacteria that do not readily grow on general-purpose media. Thus, chocolate agar is more sensitive to microbial detection than standard aerobic culture media.[19]

In addition, the gastrointestinal tract, a potential source of instrument contamination, contains many anaerobic, spore-forming bacteria that cannot be detected via aerobic culture.[16] These spores can be transmitted to subsequent animals during batch surgeries via insufficiently sterilized surgical instruments. Studies involving gastrointestinal instrument contamination should incorporate anaerobic bacterial culturing measures. *Brucella* blood agar has hemin and vitamin K1, which were shown to be needed to cultivate certain types of obligate anaerobes. It also has sodium bisulfite, which lowers the redox potential for the media to a range suitable for obligate anaerobes.[5]

Finally, ATP bioluminescence is commonly used to measure the ATP found in organic material and thus serves as an indirect measure of surface contamination.[14,20] ATP bioluminescence may indirectly detect bacterial species that do not readily grow on most growth media. While the focus of previous work has been on the presence of viable bacteria on surgical instruments, residual bacterial or other organic material may also have a negative impact on rodent health and should be removed whenever possible.[11,14]

The objectives of the present study were 2-fold. The first objective was to examine if the addition of a detergent step (that is, multienzyme detergent, neutral pH detergent, or chlorhexidine scrub) step to a GBS protocol improved the success rate of glass bead sterilization. The second objective was to optimize post-GBS validation methods via aerobic culture (sheep blood agar and chocolate agar), anaerobic culture (*Brucella* agar), and ATP bioluminescence.

Materials and Methods

Instruments. Stainless steel Adson dressing forceps and needle drivers of appropriate size for rodent surgeries were used in this study. The tips of the forceps were serrated while those of the needle drivers were smooth. Before each use each instrument was thoroughly cleaned and autoclaved. The autoclave used was routinely tested by facility personnel with biologic indicators to ensure proper function, and within each instrument package, an indicator (Thermalog Steam Chemical Integrator, St. Paul, MI) was added to confirm a successful autoclave run. The autoclave in the facility uses gravity sterilization, and the cycle used for our project was consistent with that used for surgical instruments for other procedures.

Mice. A total of 55 mice (*Mus musculus*) were used. In keeping with the 3Rs, only mice that were originally marked for planned euthanasia were used for testing. All animals were housed at an AAALAC accredited facility in accordance with the *Guide for Care and Use of Laboratory Animals* (8th edition). Mice were housed in polysulfone IVC cages (cage bottom no. 75301; Lab Products, Seaford, DE) with hardwood maple bedding (number 7090; Sani-chips; Harlan Teklad, Madison, WI). Enrichment was provided in the form of cotton squares (Ancare, Bellmore, NY). Cages were maintained on a 12:12 light:dark cycle. Temperature (68 to 79 °F [20 to 21 °C]) and humidity (30% to 70%) of rooms housing mice always remained within acceptable limits. Mice always had ad libitum access to acidified water and rodent chow (reference number 5001; Laboratory Rodent Diet, Richmond, IN). The facility colony health surveillance program was conducted on a quarterly basis. It consisted of live sentinel use with samples sent to a third party for diagnostic analysis. The excluded agents of the facility were as follows: Sendai virus, murine hepatitis virus, mice minute virus, mouse parvovirus 1 and 2, Theiler virus, epizootic diarrhea of infant mice, lymphocytic choriomeningitis virus, extromelia virus, *Mycoplasma pulmonis*, myeloblastosis associated virus 1 and 2, pneumonia virus of mice, mammary tumor virus, reovirus, Hantaan virus, *Filobacterium rodentium*, *Myobia, Mycoptes*, and *Radfordia* spp. and *Aspicularis* and *Syphacia* spp.

Twenty-four mice were male and 31 were female. The mice generally were of C57BL/6 background, though several were either C3H, FVB, or ICR. All animals originated from animal use protocols that were approved by the Texas A&M University Institutional Animal Care and Use Committee. Beforehand it was determined that mice selected did not have any genetic modifications that were immunomodulatory or would otherwise have a confounding impact on bacterial population numbers or composition. The mice were euthanized via carbon dioxide asphyxiation followed by cervical dislocation and immediately used for the study. Euthanasia was carried out in accordance with institutional standard operating procedures and the 2020 AVMA Guidelines for Euthanasia of Animals.

Surgical access to cecum. Each mouse was placed in dorsal recumbency. Rat-toothed forceps and iris scissors were used to enter the abdominal cavity. The cecum was exteriorized, and with the use of iris scissors, a small incision was made in the antimesenteric side of the cecum. The iris scissors were placed to the side, and the rat-toothed forceps were used to slightly open the cecum so the instruments to be tested could be inserted. The forceps and needle drivers were then sequentially inserted approximately 1 cm into the lumen of the cecum. After removal, the instruments then sat for 2 to 3 min to allow the cecal contents to dry and better affix to them. The same individual carried out this step for all surgical instruments and was wearing surgical gloves, a hair bonnet, and a surgical mask. This step was conducted in a dedicated space for rodent surgery with limited traffic flow in general and none while carrying out this procedure.

Instrument cleaning protocols. The cecal contents of one mouse were used to contaminate 4 sets of instruments (each set contained one forceps and one needle driver). Each set of instruments was assigned one of 4 possible cleaning protocols (below).

No instrument was reused in the study before thorough cleaning and autoclaving. After contamination with the cecal contents, each instrument tip was wiped 2 to 3 times with a clean, dry paper towel for gross debris removal. The instruments then proceeded to one of the 4 cleaning protocols.

Neutral pH detergent protocol–A working solution of neutral pH detergent (Securos Surgical Cleaner/Lubricant; Amerisource Bergen, Fickdale, MA) was prepared from concentrate as per manufacturer guidelines of one fluid ounce (30 mL) per gallon of warm water. The instruments were fully submerged for 5 min (minimum time recommended by the manufacturer) (Figure 1). Because a gallon of the working solution was produced, submerging the entire tools was feasible.

Multienzyme detergent protocol–A working solution of a multienzymatic instrument detergent (multienzyme detergent; Jorgensen Laboratories, Loveland, CO) was prepared from concentrate, as per manufacturer guidelines of one fluid ounce (30 mL) per gallon of warm water. The instruments were fully submerged in the working solution for 5 min (minimum time recommended by the manufacturer) (Figure 2). Because a gallon of the working solution was produced, submerging the entire tools was feasible.

Chlorhexidine protocol–The tips of each instrument were submerged in chlorhexidine scrub (Aspen Veterinary Resources, Loveland, CO) for 2 min (Figure 3). The 2-min time period was chosen because this is the contact time for chlorhexidine.[1] Fully submerging the entire instrument would have required a large quantity of chlorhexidine, so only the tips were submerged.

Figure 1. Instruments fully submerged in neutral pH detergent.

Figure 2. Instruments fully submerged in multienzyme detergent.

Figure 3. Instrument tips submerged in chlorhexidine scrub.

Saline protocol–With this group the tips of each instrument were briefly (1 to 2 s) placed in a small cup of sterile saline (Vedco, St. Joseph, MO) before further cleaning (Figure 4). Fully submerging the entire instrument would have required a large quantity of saline, so only the tips were submerged.

A generalized workflow from instrument contamination to instrument pretreatment is depicted in Figure 5.

After the soaking time for each group, each instrument tip was brushed with a clean toothbrush (Midwest Veterinary

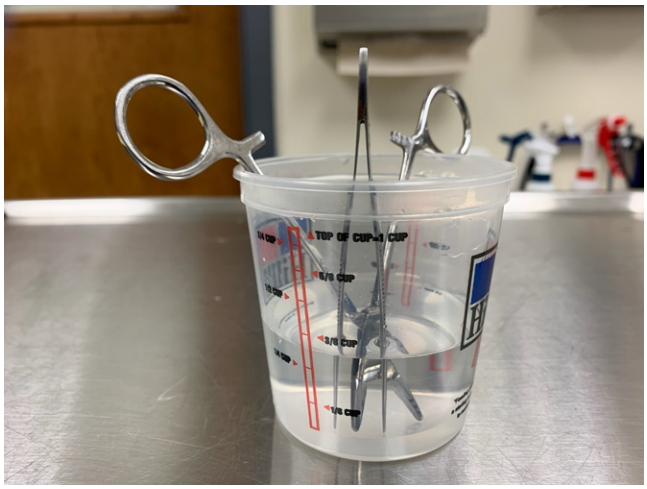

Figure 4. Instrument tips submerged in sterile saline.

Supply, Lakeville, MN) (Figure 6). All 4 sides of each tip were brushed for a total of 10 s, alternating between horizontal and vertical brushing motions. After brushing, the instrument tips were submerged in their respective cleaning solutions and rigorously moved back to further enhance debris removal. Then, all sides of each tip were washed with about 3 mL sterile saline using a syringe and 20-g needle. Afterward, each instrument was inserted into the GBS.

Glass bead sterilization. Before study initiation, a new bag of glass beads was added to the GBS (Germinator 500; CellPoint Scientific, Gaithersburg, MD). The GBS was turned on 30 min before usage. In addition to the device's green indicator light, a digital thermometer was briefly placed in the beads to ensure the temperature had reached 500 ± 50 °F (250 ± 10 °C). Bead temperatures were also taken at the midpoint and end of each testing session to ensure the working temperature consistently remained within range. As per manufacturer settings, the instruments were inserted to a depth of at least one inch into the beads. The instruments were not allowed to touch either the bottom or sides of the bead compartment. Both the needle drivers and forceps were placed into the GBS at the same time and remained inserted for 60 s. The instruments were allowed to cool for 20 to 30 s before microbial or ATP sampling. A general

Figure 6. Brushing instrument with clean toothbrush.

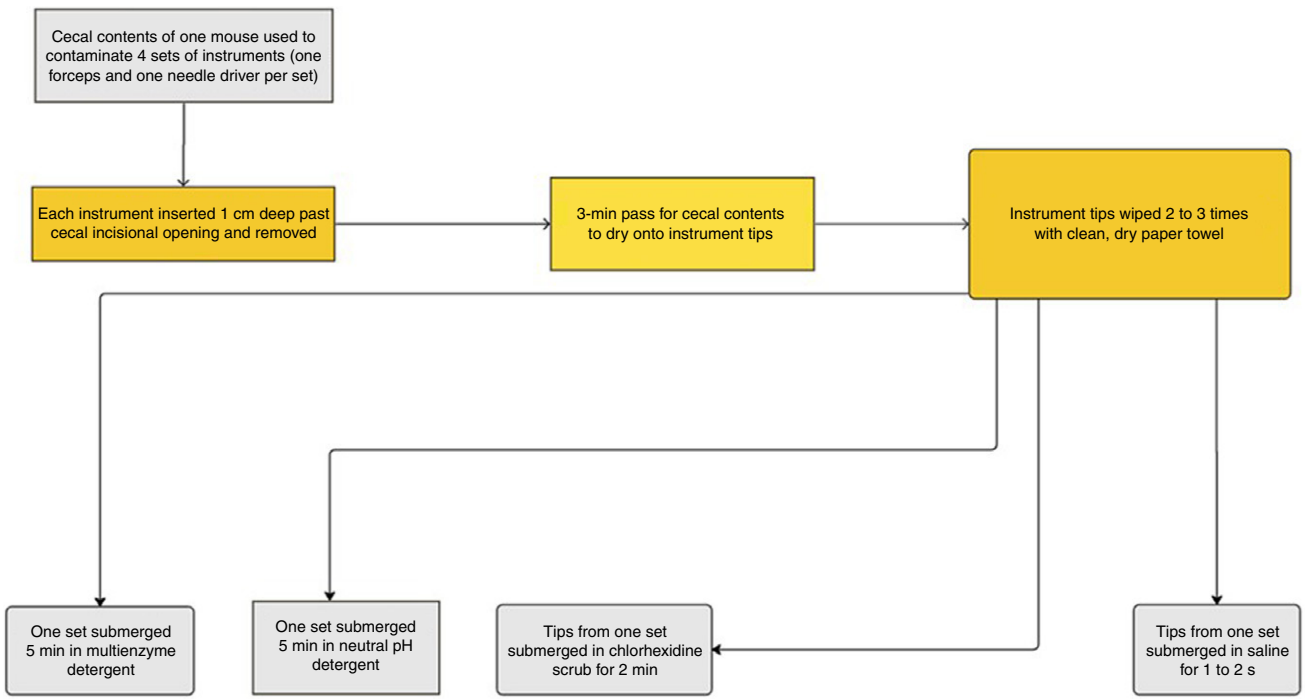

Figure 5. General workflow of instrument cecal contamination to assigned treatment protocol.

workflow of tools from being brushed with a toothbrush until glass bead sterilization is depicted in Figure 7.

Microbial and ATP sampling. A sterile cotton swab was used to collect all bacterial culture samples. All 4 sides of the instrument tip were swabbed. For all sides of the instrument tip being sampled the swab was moved vertically up and down 4 times, followed by 4 horizontal movements. The swab was rotated 90° before moving to the next side of the instrument tip. The swab was then immediately vortexed for 5 s in 1 mL of tryptic soy broth (Hardy Diagnostics, Santa Maria, CA), and 100 µL was plated onto the bacterial media plate.

For the ATP luminescence assay, the same basic sampling technique as the cotton swabs was used, but cotton swabs were substituted with PocketSwab Plus ATP swabs (Charm Sciences, Lawrence, MA). After sampling the swabs were placed into the luminometer for analysis. As a positive control, swab samples were obtained from one set of forceps and needle drivers after exposure to cecal contents, but before any cleaning or sterilization procedures.

Culture methods. The first 10 sets of instrument samples were collected for bacterial culturing onto blood agar plates (Carolina Biological Supply, Burlington, NC). For each instrument, one tip was sampled before any cleaning protocol with cecal contents still on it, and the other tip was sampled after its assigned cleaning protocol was completed. The tip sampled with cecal contents served as a positive control for each instrument. For all subsequent instruments, one tip was swabbed and plated onto either blood agar, chocolate agar (Carolina Biological Supply), or *Brucella* agar (Thermo Fisher Scientific, Waltham, MA) or subjected to ATP swabbing. The other tip of that same instrument was also swabbed and plated onto either blood agar, chocolate agar, or *Brucella* agar or subjected to ATP swabbing. No instrument had both tips either plated onto the same media or swabbed for bioluminescence testing. Some instruments only had one tip sampled due to the varying availability of culture media at the time of sampling (Figure 8). All bacterial plates were incubated at 37 °C for 48 h. Plates were noted as positive or negative for bacterial growth.

For anaerobic culturing, the *Brucella* agar plates were placed into canisters with locking clamp lids with silicone seals (Clear Canister Set; Walmart, Bentonville, AR). An anaerobic gas pack (Fisher Scientific, Hampton, NH) was placed into each canister

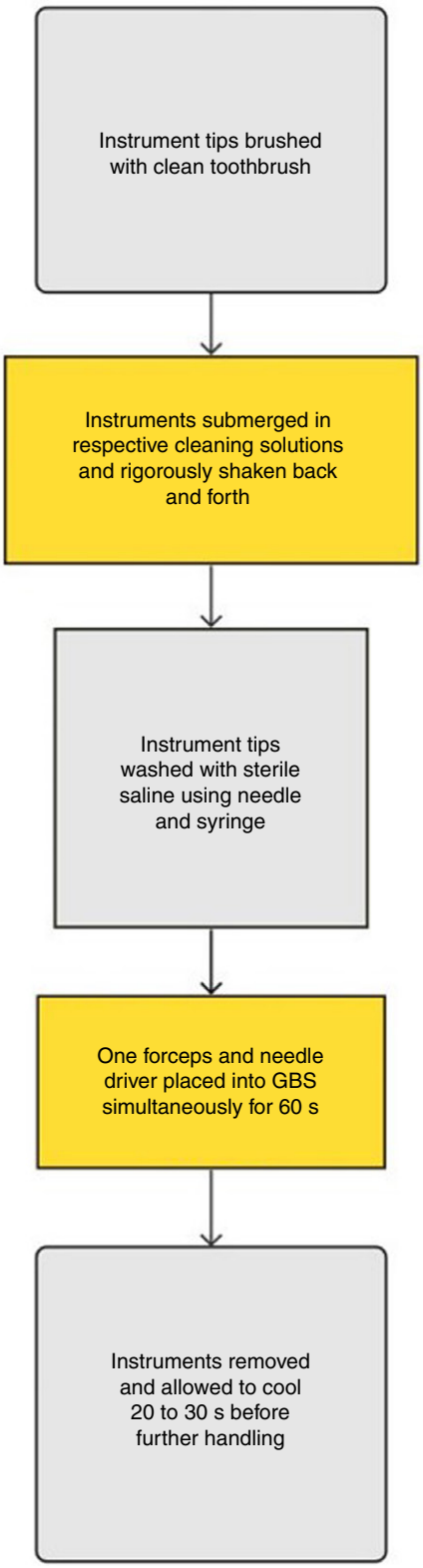

Figure 7. General workflow of instruments from assigned pretreatment protocols until glass bead sterilization.

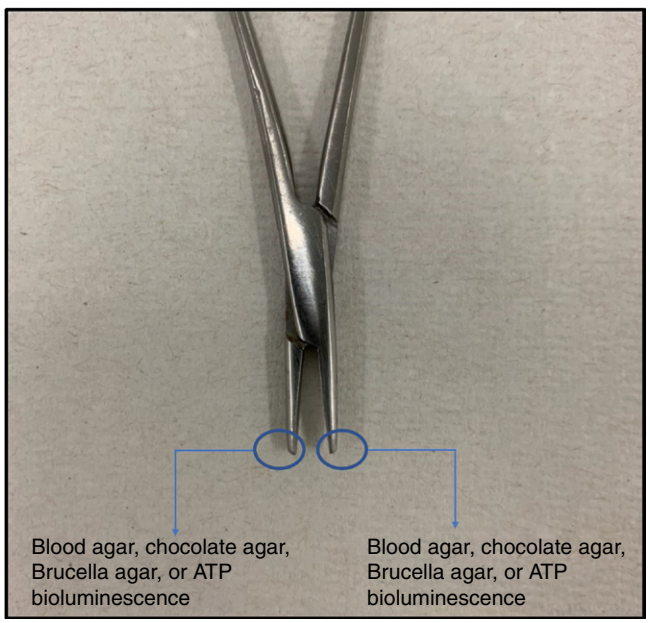

Figure 8. Schematic of instrument tip swabbing for subsequent bioluminescence or bacterial culture testing. Separate sterile swabs were used for different instrument tips. No instrument had either both tips swabbed and plated onto the same media or both tips sampled for bioluminescence. For some instruments only one tip was sampled due to varying availability of culture media at the time of sampling.

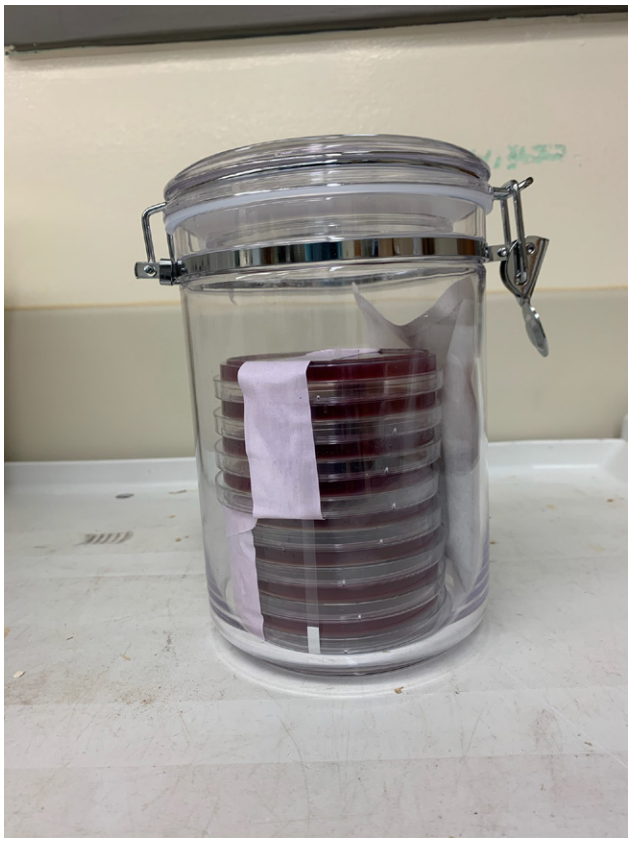

Figure 9. Anaerobic chamber with *Brucella* agar plates shown. Indicator strip in center turned white, indicating anaerobic conditions were present in jar. Anaerobic gas pack also seen to right of agar plates.

along with an anaerobic indicator strip (Becton, Dickinson, Sparks, MD) to ensure anaerobic conditions were attained (Figure 9). All bacterial plates were incubated at 37 °C for 48 h Plates were noted as positive or negative for bacterial growth.

To verify culture media integrity, one plate from every new sleeve of bacterial media was inoculated to serve as a positive control. A forceps tip was exposed to cecal contents and was swabbed as described above. The cotton swab was vortexed for 5 s in 1 mL of tryptic soy broth, and 100 µL was plated onto the agar plate. A positive culturing result was used as a proxy to determine the integrity of the rest of the agar plates in the sleeve.

Analysis and statistics. From an analysis standpoint, the likelihood of an instrument tip yielding positive culturing or bioluminescence results was evaluated. Given that a single instrument had tips that were analyzed with different bacterial media or bioluminescence, we did not analyze on the whole instrument level. In total, there were 23 needle driver tips and 23 forceps tips per cleaning protocol that were plated onto sheep blood agar. This amount was 20 for both needle driver and forceps tips plated onto chocolate agar, 20 for *Brucella* agar, and 21 for ATP bioluminescence (Table 1).

To assess differences in bioluminescence values, we conducted both dichotomous and continuous comparisons. Dichotomous comparisons examined whether any bioluminescence was detected for each instrument and were conducted via a one-sided Fisher exact test for most comparisons and a 2-sided for the general comparison of bioburden between needle drivers and forceps. Continuous comparisons examined relative light unit (RLU) levels and were conducted via a nonparametric Kruskal-Wallis test (adjusted for ties). Statistical comparisons were conducted in STATA version 14.2 (StataCorp, College Station, TX). The cutoff for statistical significance was $P < 0.05$.

Results

All positive control plates had bacterial growth, producing a mat of bacterial growth that did not have countable colonies. Out of the tips of the 23 sets of needle drivers and forceps exposed to all 4 decontamination treatment protocols and plated onto sheep blood agar, no positive results were yielded. Two forceps tips from the saline group were positive for bacterial growth on chocolate agar. One forceps tip from the saline group yielded growth on *Brucella* blood agar. No other instrument tips across all treatment groups yielded growth on each of these media. Due to the low levels of plates with positive culturing results, statistics to compare each group could not be done. Culturing results are summarized in Table 2.

Both the control forceps and needle driver tips tested positive with ATP swabbing (1,786,885 and 1,781,179 RLU, respectively). No instrument tip from the neutral pH detergent group tested positive with ATP swabbing. One needle driver tip from the

Table 1. Number of tips from different instruments exposed to each treatment protocol and subsequent media for bacterial culturing or ATP bioluminescence

	Multienzyme detergent		Neutral pH detergent		Chlorhexidine scrub		Saline	
	Forceps	Needle drivers	Forceps	Needle drivers	Forceps	Needle drivers	Forceps	Needle drivers
Sheep blood agar	23	23	23	23	23	23	23	23
Chocolate agar	20	20	20	20	20	20	20	20
Brucella agar	20	20	20	20	20	20	20	20
ATP bioluminescence	21	21	21	21	21	21	21	21

Table 2. Number of forceps and needle driver tips from each treatment group that yielded bacterial growth on each bacterial medium

	Multienzyme detergent		Neutral pH detergent		Chlorhexidine		Saline	
	Forceps	Needle drivers	Forceps	Needle drivers	Forceps	Needle drivers	Forceps	Needle drivers
Sheep blood agar	0/23	0/23	0/23	0/23	0/23	0/23	0/23	0/23
Chocolate agar	0/20	0/20	0/20	0/20	0/20	0/20	2/20	0/20
Brucella agar	0/20	0/20	0/20	0/20	0/20	0/20	1/20	0/20

multienzyme detergent group produced positive results (133 RLU). Four instrument tips from the chlorhexidine group had positive ATP swabbing results. The mean for instrument tips testing positive was 2,171.25 RLU (range: 661 to 4,293 RLU). Two of the instrument tips came from needle drivers (mean = 3,603 RLU; range: 2,913 to 4,293 RLU) and the other 2 were from forceps (average: 739.5 RLU; range: 661 to 818 RLU). Four instrument tips from the saline group tested positive for ATP swabbing. All 4 instrument tips were from needle drivers; the mean RLU of these instrument tips was 29,460.25 RLU, with a range of 376 to 85,401 RLU.

In either dichotomous ($P = 0.17$) or continuous ($P = 0.09$) comparison, there was no statistically significant difference between needle driver and forceps tips in terms of organic contamination (Tables 3–5). In dichotomous comparisons of needle driver tips, there was no significant difference in organic contamination presence in those with pretreatment with any cleaner compared with those with only saline ($P = 0.06$). Similarly, there was no significant difference observed between saline and multienzyme detergent ($P = 0.18$), neutral pH detergent ($P = 0.05$), or chlorhexidine ($P = 0.33$). There were no statistically significant differences in any comparison with forceps tips, either in aggregate or when comparing individual instrument tips.

In a continuous comparison of needle driver tips, pretreatment with any of the pretreatment options resulted in a statistically significantly decreased RLU burden when compared with only saline exposure ($P = 0.034$). For needle driver tips, when comparing individual cleaners, there was no statistically significant difference in RLU burden between saline and multienzyme cleaner ($P = 0.13$) or chlorhexidine ($P = 0.34$). However, pretreatment with a neutral pH detergent did result in a decrease ($P < 0.05$) when compared with saline. There were no statistically significant differences in any comparison with forceps tips, either in aggregate or when comparing individual instrument tips.

Table 3. Comparison of observed bioluminescence for treatment groups in needle driver tips

	Mean RLU	Number of needle driver tips with bioluminescence detected
Saline	5,611	4/21
Multienzyme detergent	133	1/21
Neutral pH detergent	0	0/21
Chlorhexidine scrub	70	2/21

Table 4. Comparison of observed bioluminescence for treatment groups in forceps tips

	Mean RLU	Number of forceps tips with bioluminescence detected
Saline	0	0/21
Multienzyme detergent	0	0/21
Neutral pH detergent	0	0/21
Chlorhexidine scrub	343	2/21

Discussion

Each of the tested GBS protocols represents a major improvement in our understanding of how best to sterilize rodent surgical instruments exposed to heavy microbial contamination. In one study,[10] after cecal contamination, there was only a 57.1% chance that glass bead sterilization would yield no bacterial growth using aerobic culture methods on general-purpose media. No instrument tips in our study yielded bacterial growth on general-purpose media after glass bead sterilization, including those from the saline group. Furthermore, the 3 pretreatment groups yielded no growth on chocolate agar, which is more sensitive than sheep blood agar, and *Brucella* agar, which covers anaerobic organisms that would not grow on sheep blood agar. Interestingly, even the saline-only GBS group largely outperformed the treatment protocols in prior studies. In addition to no bacterial growth found on sheep blood agar, even on the other 2 media types, the overwhelming number of plates did not yield bacterial growth. In one study,[10] manufacturer guidelines were followed, which called for instruments to be washed until visible debris could no longer be seen. However, in our study, even the saline-only group had its instrument tips wiped with a paper towel before being rinsed with saline. In addition, these instrument tips were also brushed with a toothbrush before using the GBS. These additional steps to remove gross debris on the instruments likely played a significant role in improving instrument sterilization.

In addition to aerobic culturing onto general-purpose media, this study more thoroughly analyzed the presence of contamination on instruments after each GBS protocol with other culturing media and conditions and ATP swabbing. Because of this, we can conclude from our results with more confidence and finely distinguish the relative efficacy of each GBS protocol. Though generally effective, the saline group did yield several positive culture results on nongeneral-purpose media. These results would not have been generated without the use of such media. Future studies evaluating GBS efficacy should incorporate more than general-purpose media alone to test sterility.

The usage of ATP swabs has also helped differentiate the relative effectiveness of each cleaning protocol. Instrument tips from the saline group were not significantly more likely to test positive than those from the pretreatment protocols. Several differences were close though, including the likelihood of needle driver tips from the saline group testing positive compared with needle driver tips from any of the pretreatment protocols ($P = 0.06$). Another example is the likelihood of needle driver tips from the saline group to test positive compared with needle driver tips from the neutral pH detergent group ($P = 0.05$). Significant differences were seen in the average RLU levels for instrument tips that tested positive between different treatment groups. Needle driver tips from the saline group produced higher RLU levels compared with needle driver tips from all pretreatment groups combined. Needle driver tips from the saline group also produced higher RLU levels compared with those from the neutral pH detergent group. It is not immediately clear why needle driver tips were more likely to have such differences in bioluminescence compared with forceps tips. Theoretically, the grooves on the forceps tips should make debris

Table 5. Summary of significant differences in bioluminescence mean RLU levels between instruments from different treatment groups

	Instrument	Significance	Higher mean RLU
Use of any pretreatment group (multienzyme detergent, neutral pH detergent, or chlorhexidine scrub) compared with saline only	Needle drivers	$P = 0.034$	Saline only
Neutral pH detergent compared with saline only	Needle drivers	$P < 0.05$	Saline only

removal more difficult, leading to increased organic contamination. This also runs in contrast to the culturing results, where bacterial growth was only yielded from forceps tips. Whatever the reason for this difference may be, this does highlight the need to be thorough in cleaning both instruments with flat and corrugated surfaces.

An important limitation of this study is the sample size employed. While measures of effect for certain comparisons compared with the saline-only group may not have been statistically significant in dichotomous comparison, the strong odds ratio (OR) observed in the likelihood of needle driver tips from the saline group to test positive for bioluminescence compared with needle driver tips from all other pretreatment groups (OR = 4.71) indicates an effect. Simply cloning the dataset (increasing sample size while holding the OR constant) would result in statistical significance for these comparison groups. This may represent a direction for future study. In addition, a larger sample size may allow for a more rigorous analysis of the differences in bacterial culturing values.

A future direction of this study would be to repeat it but with multiple surgical events. To simulate rodent batch surgeries, the instruments would be exposed to cecal contents and sterilized several times before bacterial culture and ATP bioluminescence testing. Given that one study[10] demonstrated that there is a decline in efficacy over time as instruments were reused and subjected to glass bead sterilization, it would be important to repeat our study in a likewise manner. In addition, this would more closely resemble circumstances where researchers conduct rodent batch surgeries and must resterilize their instruments several times. In addition, while obtaining postprocedural contamination results is important, ultimately this data must be correlated with either clinical or subclinical infection in living animals. Although the saline group in this study yielded some positive culture results and the highest bioluminescence results, this procedure may not lead to any significant health or experimental differences compared with the other cleaning groups in this study.

Although the methodology used in this study for microbial isolation and culturing is standard, transferring 100 µL of broth from the 1-mL the cotton swab was submerged in onto an agar plate does yield a one-in-ten dilution. Because of this, the bacteria may be present but at levels below the level of detection for this methodology. Strictly liquid media-based methods determining positive compared with negative culture results are more sensitive but are also more likely to be undercut by environmental contamination.

Typically, in rodent surgery, the skin is aseptically prepared to minimize the microbial load at the incisional site. However, in our procedures, we did not take such precautions when surgically accessing the cecum. When the cecum was exteriorized the portion cut with iris scissors did not come in contact with the skin. In addition, rat-toothed forceps held open the cecum, and instrument tips were able to be inserted into the lumen so that they did not have any contact with the skin. Despite the lack of skin preparation, each of the 4 treatment protocols still represents an improvement over the previous protocols described. Given this, theoretically, for an actual rodent surgery with enhanced aseptic technique, these protocols should still perform well.

One potential concern with the repeated use of chlorhexidine scrub as a pretreatment option is the chance of instrument tip erosion over time.[1] This in turn could decrease the utility and lifespan of these surgical instruments over the course of many surgical events. While the chlorhexidine scrub was washed off with sterile water before sterilization, potentially some may still remain. This could be a point of consideration for further investigation into the usage of chlorhexidine scrub in GBS protocols.

Although more work is needed to further refine GBS protocols, the results from this study demonstrate promise for using either of the detergents or chlorhexidine scrub to augment instrument sterilization for rodent batch surgeries. Furthermore, the use of testing modalities beyond aerobic culture using general-purpose media to assess instrument sterility is also needed to more effectively determine the efficacy of any GBS protocol moving forward.

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Conflict of Interest

The authors have no competing interest to declare.

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Author Contribution

All authors contributed to study conception and design. Material preparation, data collection, and analysis were performed by Keith Lewy and Timothy A Erickson. The first draft of the manuscript was written by Keith Lewy and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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