Sterility Maintenance Study: Dynamic Evaluation of Sterilized Rigid Containers and Wrapped Instrument Trays to Prevent Bacterial Ingress

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\textbf{Background:} Sterilized packaging systems are designed to maintain the sterility of surgical instruments and devices from the time of sterilization until use. This study evaluated the effectiveness of rigid containers versus wrapped instrument trays, sterilized using North American sterilization protocols, to maintain a sterile internal environment poststerilization when challenged with aerosolized bacteria under dynamic environmental conditions.

\textbf{Methods:} Using a custom aerosol chamber, 111 rigid containers of various durations of use (unused, used <5 years, used 5-9 years) and 161 wrapped trays using 3 grades of sterilization wrap were challenged with \(10^{2}\) colony-forming units per liter of air containing aerosolized \textit{Micrococcus luteus} with an air particle size of 1 mm, while simultaneously experiencing air volume changes due to vacuum cycles—two 1-psi cycles, three 0.7-psi cycles, and three 0.4-psi cycles—to simulate air exchange events occurring during the sterilization, transportation, and storage of sterilized instrument trays in health care facilities.

\textbf{Results:} Of 111 rigid containers tested, 97 (87\%) demonstrated bacterial ingress into the container. Of 161 wrapped trays, 0 (0\%) demonstrated bacterial ingress into the tray. Contamination rates of rigid containers increased significantly with increasing duration of use.

\textbf{Conclusions:} In this study using a dynamic bacterial aerosol challenge, sterilized wrapped trays demonstrated significantly greater protection than sterilized rigid containers against the ingress of airborne bacteria.

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Approximately 300,000 surgical site infections (SSIs) occur annually in US hospitals, resulting in an estimated 9000 attributable deaths.\textsuperscript{1-3} Maintaining a sterile environment in the operating room (OR) is essential for SSI prevention and requires that all surgical instruments and/or medical devices be sterile before use. Various methods (eg, steam autoclave, ethylene oxide) are traditionally used for sterilizing surgical implements, whereas sterilization packaging systems (SPSs), including synthetic disposable wrap and rigid containers, are used to enclose and maintain the sterility of these instruments or devices. The potential combination of SPS material breaches and/or malfunctions with air exchanges that occur poststerilization make it unrealistic to assume that previously sterilized surgical instruments and devices always remain sterile until their use in the OR.

When SPS are functioning properly, room air enters either through filters or valves in rigid containers or through the wrap material itself for wrapped trays, thereby preventing the ingress of airborne bacteria and maintaining content sterility. However, damage, misuse, or poor construction of SPSs can create barrier breaches through which bacteria could enter without being stopped by filter fibers or valves. For rigid containers, breaches could result from poorly joined, oxidized, cut, or compressed gaskets; gaps caused by mismatched lids and bases; loose filter...
retainers; loose rivets or fasteners; and other breaches due to misuse, damage, or age-fatigue. In addition, the aluminum body of most rigid containers can be degraded by incompatible detergents, incorrect pH, or repeated metal-on-metal rubbing during cleaning. For wrapped trays, poor fabric design, treatments, or construction; improper folding techniques; and/or tears can result in barrier breaches. These SPS breaches potentially create entry points for airborne bacteria that may ingress into the SPS owing to air exchanges resulting from normal hospital operations, such as cool down after steam sterilization, air pressure and temperature changes in storage areas, and transport between departments and hospitals.

Several previous studies have raised questions about the ability of SPSs to maintain the sterility of surgical instruments, devices, and implants until use in the OR. To properly evaluate the sterility maintenance capability of a specific SPS, a dynamic biological aerosol test that creates air exchanges through the SPS is required. Junghanns et al. challenged rigid containers containing nutrient agar plates with a Bacillus subtilis aerosol while drawing a vacuum through a tube inserted through the side of the containers. Although the authors reported that all 22 containers tested demonstrated >99.99% barrier efficiency, they measured neither the bacterial aerosol concentration nor the particle size. In addition, the test containers were damaged to accommodate the vacuum tube, sterilized using European sterilization conditions, and opened post-sterilization to insert the nutrient agar plates. Dunkelberg and Fleitmann-Gende used a bioaerosol chamber to expose sterilized rigid containers holding sterile nutrient agar trays to aerosolized Saccharomyces cervisiae (yeast), while simultaneously exposing 24 vacuum cycles of 70 hPa (1 psi) over a 2-hour period. The aerosolized Saccharomyces entered 173 of the 216 rigid containers tested (80.1%). Although Dunkelberg and Fleitmann-Gende sterilized their nutrient agar inside the rigid containers and avoided damaging the containers, they did not measure microbial aerosol concentration or particle size. Moreover, the air exchange regimen was relatively aggressive, and the rigid containers were sterilized using European sterilization conditions.

In the present scientific study, we evaluated the sterility maintenance capability of sterilized rigid containers and sterilization wrap, using a test method similar to that described previously, but with notable differences. In contrast to the previously described sterility maintenance testing methods, in this work both rigid containers and instrument trays wrapped with disposable sterilization wrap were challenged, without intentional modifications, by an evenly dispersed aerosol of a specific particle size containing a known concentration of bacteria while simultaneously being subjected to less rigorous air exchanges. The test bacterium, Micrococcus luteus, is comparable in size and shape to Staphylococcus aureus and coagulase-negative staphylococci, which together represent more than 43% of SSIs in the United States. This is the first dynamic assessment of the barrier protection provided by SPSs against aerosolized bacteria using North American sterilization protocols.

MATERIALS AND METHODS

Test microorganism

*M. luteus* (ATCC 4896) is a Gram-positive aerobic cocci ranging in size from 1.0 to 1.8 μm. For the present study, *M. luteus* was propagated by suspending a 1-mL freeze-dried aliquot in sterile tryptic soy broth (TSB), and then transferred to 50 mL of sterile TSB in a 250-mL Erlenmeyer flask under aseptic conditions. The Erlenmeyer flask was placed on a shaker incubator (model I2400; New Brunswick Scientific, Enfield, CT) at 220 rpm and incubated for 18-24 hours at 30°C. After incubation, the bacterial suspension was centrifuged at 3,600 × g for 10 minutes to remove cellular debris. The supernatant was discarded, and cells were resuspended in sterile deionized water (sdH2O) in equal volume to the supernatant.

Each bacterial propagation was titered in triplicate to determine the viable cell concentration in phosphate-buffered saline (PBS). A volume of 0.1 mL of each dilution was pipetted onto a sterile TSA plate, then spread using a sterile L-shaped spreader. Inoculated plates were incubated at 30°C for 48-72 hours. A mean bacterial concentration of ∼1 × 10^6 colony-forming units (CFU)/mL was achieved. Once titered, the bacterial suspensions were divided into 1-mL aliquots and frozen at −20°C. In preparation for each aerosol challenge, a 1-mL aliquot of frozen *M. luteus* was thawed at room temperature and diluted to a concentration of 1 × 10^6 CFU/mL in sdH2O.

Test chamber description

The custom test chamber, designed and validated by Applied Research Associates (ARA; Panama City, FL), measures 1.0 m W × 1.1 m H × 0.8 m D and is constructed of 0.635-cm thick aluminum (Fig 1). The front face of the chamber, measuring 1.0 m W × 1.1 m H, serves as a hinged door and is secured by sixteen 1.27-cm bolts and sealed with a 0.32-cm-thick silicone gasket. Inside the chamber, the structure is supported by three 2.54 cm aluminum support bars, oriented along the x-, y-, and z-axes for reinforcement during vacuum cycles. Four wire shelves are placed in the chamber along with 2 fans, positioned midline in the front and back of the chamber to promote particle distribution. A manifold made of stainless steel tubing (1.27 cm o.d., 0.09 cm-thick walls) connects to 2 Swagelok-fitted ports at the bottom of the chamber and the main components of the vacuum system.

To generate a vacuum inside the chamber, a 1.1-CFM compressor (Cole-Parmer, Vernon Hills, IL) with a high-efficiency particulate air (HEPA)-filtered exhaust is connected to the stainless steel manifold, and its vacuum rate set by a 0.635-cm pipe thread needle valve. To release the vacuum, a solenoid valve (ASCO, Florham Park, NJ) controls the rate of the chamber exhaust with a needle valve connected to a HEPA filter. Both the vacuum compressor and solenoid valve are controlled using LabVIEW 2012 version (National Instruments, Austin, TX). A temperature/humidity probe (Monarch...
Instruments, Amherst, NH) also interfaces with the LabVIEW program and provides continuous monitoring.

A 6-Jet Collison nebulizer (BGI, Waltham, MA) was used to generate the test aerosols, operating at 20 psi and generating an air flow output of approximately 12 L/min. The compressor for the Collison nebulizer is connected to the stainless steel manifold, and thus draws air from the chamber. The air passes through the nebulizer and is directed back into the chamber via two 0.953-cm ports on the top of the chamber. This configuration prevents a net effect on the overall vacuum level of the chamber when the aerosol is added to the chamber. For aerosol sampling, five 0.953-cm ports are located on the top of the chamber—one in each corner and one in the center. An additional port is incorporated into the top of the stainless steel manifold for viable sampling using an All-Glass Impinger (AGI-30; Ace Glass, Vineland, NJ) containing 20 mL of PBS, and drawing air at a flow rate of approximately 12.5 L/min. The exhaust air from the compressor connected to the AGI-30 is redirected back into the chamber via an additional 0.953-cm port in the top of the chamber, thus generating no net effect on the overall vacuum level of the chamber during viable sampling.

Test packages

A mix of used rigid containers obtained from the in-use inventories of various health care facilities (9 acute care hospitals, 2 teaching hospitals, 1 children’s hospital, 1 ambulatory surgery center, and 1 government hospital) representing geographic diversity throughout the United States and Canada, together with new containers purchased from the open market, were tested for this study. Various container designs were evaluated, including plastic and aluminum lids, reusable polytetrafluoroethylene and disposable filters, and those using a valve system. For sterilization wrap, multiple lots from several grades of single-use polypropylene wrap were obtained from a single manufacturer. Grades of sterilization wrap indicate fabric weight, with lower fabric weights used for lighter instrument trays and higher fabric weights for heavier instrument trays.

For the purposes of this study, test packages were categorized based on duration of use (Table 1). For used containers with less than 5 years of use, the mean duration was 3.1 ± 0.7 years. For containers with 5+ years of use, the mean duration was 5.9 ± 1.2 years.

Test package sterilization

Test packages were transported to the Sterile Processing Department of Bay Medical Center/Sacred Heart Health System (Panama City, FL), and sterilized in an AMSO Eagle 3000 autoclave (Steris, Grand Prairie, TX) using a 4-minute, 132°C prevacuum cycle followed by a 30-minute drying period. An Attest Rapid 5 Steam Plus Test Pack (3M) containing both a biological and chemical indicator was included in all autoclave cycles. Rigid containers and wrapped instrument trays were sterilized simultaneously in the same autoclave during the same run, ensuring that wrapped trays were located above rigid containers.

After the 30-minute drying period, test packages were kept in the autoclave for an additional 30 minutes to cool down, then removed and placed on another cooling rack in the Sterile Processing Department for an additional 30-60 minutes until cool to the touch. Test packages were then placed in new sterility maintenance covers and transported back to the ARA facility.

On arrival, test packages were removed from the sterility maintenance covers and placed inside the test chamber. Once the chamber door was secured, the chamber was flushed with HEPA-filtered air for 30 minutes to remove ambient air particles. An aerodynamic particle sizer (APS; APS Spectrometer 3321; TSI, Shoreview, MN) was subsequently used to determine the background level of air particles in the chamber. A baseline level of <0.1 particle/cm³ was required to proceed.

Dynamic bioaerosol test

The objective of this study was to compare the ability of sterilized rigid containers and wrapped trays to resist ingress of bacteria. Table 2 provides examples of dynamic environmental events, including poststerilization cool down, storage, and transport, that
SPSs and contained sterilized items can experience change after sterilization.

For each test, 8 sterile SPSs were challenged simultaneously. A mix of rigid containers and wrapped trays was used, if available, and rotated based on location between each test. Before each test run, the chamber system was assessed for leakage by drawing a 1-psi vacuum and holding for 15 minutes. If the system demonstrated >0.1 psi loss during the hold period, a leak was assumed to have occurred.

Once the chamber passed the leak test, a 1-ml aliquot of frozen M. luteus was thawed at room temperature, and aseptically diluted in sdH2O to a concentration of 1 x 10^8 CFU/mL into a final volume of 30 mL in a 6-jet Collison nebulizer. The Collison nebulizer was secured to the chamber and run for 1-5 seconds. The APS was used to determine the particle size distribution (PSD) to ensure that the median diameter of the PSD was 1 ± 0.1 μm.

Next, the chamber was engaged in a sequential series of vacuum cycles over a 70-minute period, including two 1.0-psi cycles, three 0.7-psi cycles, and three 0.4-psi cycles, to mimic normal environmental conditions (Table 2). The Collison nebulizer was pulsed every other cycle to maintain the aerosol concentration throughout the test. Viable aerosol samples were obtained throughout each test run using AGI-30 impingers (Ace Glass), each containing 20 mL of PBS. APS samples were obtained in unison with viable samples. The target viable particle concentration was 10^2 CFU/L.

At the end of each test run, the chamber was flushed with HEPA-filtered air for >1 hour to remove the aerosol particles inside the chamber. Then impinger samples were serially diluted in PBS, plated on TSA plates, and incubated at 30°C for 48-72 hours.

Post-test processing of test packages

Once the chamber had flushed for ~1 hour, the APS was used to determine the particle concentration within the chamber. Once the particle concentration reached baseline levels, the chamber was opened, and each test package was transferred to a storage rack in a fume hood. Rigid containers and wrapped sterilized instrument trays were then wiped down with hypochlorite wipes (Hype-Wipe; Current Technologies, Crawfordsville, IN) or benzalkonium chloride decontamination. Control tests were performed on triplicate samples of both wrapped trays and rigid containers to ensure that the disinfecting wipes did not affect the viability of bacteria inside the test packages (if present); no loss in viability was observed. Test packages were allowed to sit for >15 minutes to ensure adequate time for antimicrobial activity.

Double gloves were donned, and test packages were transferred to a biological safety cabinet and opened in the sterile field. Then the external pair of gloves was donned, and a new pair was donned. A foil pack of 9 sterile forceps and 9 TSA plates were placed in the biological safety cabinet. Each membrane in the test package was removed with a separate sterile pair of forceps and placed on an individual TSA plate. Plated membranes were incubated at 30°C for 48-72 hours. After incubation, bacterial colonies were observed and enumerated.

Data analysis

A 2-tailed, unpaired t test was used to compare the contamination levels between rigid containers and sterilization wrap. One-way ANOVA with Tukey’s posttest was used to compare the sample populations based on duration of use.

RESULTS

Test parameters

For this study, we selected the lowest bacterial challenge concentration possible that still provided a consistent viable quantity measured by the AGI-30 aerosol samplers. The mean viable aerosol concentration was 2.55 ± 1.16 × 10^2 CFU/L, and particle size was maintained at 0.99 ± 0.04 μm. The test chamber temperature ranged from 20.3 to 25.4°C, and relative humidity ranged from 25.2% to 59.0%. Low variability in vacuum cycling was observed among all 59 runs.

Bacterial ingress levels

Out of 111 rigid containers tested, 14 (12.6%) had no bacterial ingress, 25 (22.5%) had ingress of 1-9 CFU, 52 (46.8%) had ingress of 10-99 CFU, and 20 (18%) had ingress >100 CFU (Fig 2). All 161 wrapped trays (100%) had no bacterial ingress. The bacterial ingress rate of all rigid containers was significantly higher than that of all wrapped trays (P < .0001), even when comparing only unused rigid containers with all wrapped trays (P < .0001).

Contamination found in rigid containers based on duration of use is shown in Figure 3. The mean level of bacterial ingress for rigid containers with 5-9 years of use was significantly higher than unused rigid containers (P = .4).

DISCUSSION

The purpose of this study was to evaluate the ability of 2 types of SPS—rigid containers having various durations of use and sterilization wrap—to prevent bacterial ingress while being challenged by air exchanges that represent dynamic events in the health care setting in the presence of a known viable airborne contaminant. Existing test methodologies instead use a static bioaerosol challenge for evaluating the barrier efficacy of SPS; however, this approach does not simulate the multiple air exchanges that will occur during transport, storage, and so on. For this study, other environmental parameters (eg, temperature, relative humidity) were designed to simulate the standard conditions experienced by SPS in a typical health care setting. Within the context of the test methodology of this study, the sterilization wrap provided better barrier protection against airborne bacterial contamination than rigid containers.

For this study, bacterial ingress was common with rigid containers poststerilization (89%). Some of the rigid containers tested,
even though identified and deemed “acceptable” and “in use” by the supplying health care facility, had loose filter housings, mismatched lids/bottoms, or dents/nicks on lids/bottoms. In addition, the seals of the rigid containers aged and lost some elasticity compared with new seal material, and sustained nicks, cuts, or matched lids/bottoms, or dents/nicks on lids/bottoms. In addition, the supplying health care facility, had loose use.

The sterilized instrument trays enclosed in sterilization wrap demonstrated no detectable bacterial ingress. Unlike rigid containers, these products are single-use items, negating issues regarding age-based loss of performance. Although sterilization wrap products must be inspected for tears and holes before and after use, they are much less complicated devices than rigid containers, which have numerous seals, filters, rivets, brackets, and latches that can allow bacterial ingress poststerilization. In addition, some wrap products are treated to create electret properties that enhance small particle capture. Different grades of sterilization wrap represent various levels of thickness and durability are used in the health care setting, based on the weight of the tray to be wrapped. No difference was found between the contamination levels of the different wrap grades; all demonstrated no detectable bacterial ingress.

In accordance with standard hospital operations, various sterile indicators were used to validate and monitor the sterilization conditions used in this study. Although these indicators demonstrate that an SPS has been properly sterilized, they are not intended to monitor the poststerilization condition of the SPS before its use in the OR. All of the indicators used as part of this study indicated proper sterilization, yet M luteus contamination was still found in 87% of rigid containers. The clinical significance of these findings is not clear, given that the endpoint of this study was to measure bacterial contamination rates, not SSI rates, of SPSs in use. However, the term “sterile” is defined as no bacteria or microorganisms present, and by this definition, if a single organism ingresses into an SPS poststerilization, then the entire contents are to be considered nonsterile.

Limitations of this study must be discussed to put the resultant data into context with the test methodology. The aerosol challenge concentration is expected to directly influence the contamination level of the challenged devices. For this study, we selected the lowest viable challenge concentration possible that still provided a consistent measurable endpoint. Bacterial aerosol concentration in a health care facility was measured by Greene et al., who found an aerosol concentration of ~2 CFU per liter of air, which is ~100-fold lower than the challenge concentration used in this study. Assuming a linear relationship, if the challenge concentration of this study were lowered by 100-fold, then 18% of the rigid containers would have still been contaminated. A follow-up study was performed on a limited number of SPSs (3 unused containers, 2 containers of <5 years of use, and 2 wrapped sterilization instrument trays) using a challenge concentration of $2 \times 10^4$ CFU/L. Three of the 5 (60%) rigid containers showed bacterial ingress, whereas the 2 sterilized wrapped instrument trays showed no bacterial ingress. The challenge concentration for this follow-on experiment was difficult to quantify accurately, but even given the uncertainty, it was clear that the challenge concentration was significantly reduced and that 60% of the rigid containers still demonstrated bacterial ingress.

Another limitation of this study is how well the simulated air exchanges compare with actual air exchanges experienced in health care settings. The pressure differentials used to challenge the SPSs for this study were defined based on the recommendation of using 0.4- to 1.0-psi pressure differentials, using significantly less aggressive challenges than previously reported, and ensuring that the data from this study could be appropriately compared with previous research. No studies could be found in the open literature that measured actual pressure differentials resulting from many of the dynamic environmental events occurring in the health care setting, as listed in Table 2; however, the largest air volume exchange an SPS could experience likely will result from the temperature differential occurring when an SPS is cooling after steam sterilization. According to ANSI/AAMI ST 79, the cool down period begins within the sterilizer chamber once the chamber door is open at the end of the cycle. Using the recommended room temperature for a sterile processing department (24°C), and the mean autoclave temperature once the cycle is complete based on the temperatures recorded in this study (53°C), a combination of Boyle’s law and Charles’ law can be applied to provide an estimate for the air exchange volume that an SPS will experience resulting from the autoclave cool down. Based on these calculations, the air volume exchange (0.3 L) correlates to a pressure difference of 1.6 psi, which is higher than the maximum pressure differential used in this study.

CONCLUSION

In summary, our data indicate that sterilization wrap provided greater protection against airborne bacterial ingress compared with rigid containers within the context of this study. Sterilization wrap products are simple devices that are always used as new products, and are flexible and conformable around the instrument tray. Rigid containers are complex devices with multiple seals and filters held by retention plates or valves that can be damaged, and demonstrate loss of performance with age and use. Our results indicate that the barrier efficacy of rigid containers may diminish over time. Although guidelines for the inspection of rigid containers have been defined, our data suggest the need for a greater level of attention to ensure the performance of rigid containers as they age. In addition, some rigid container manufacturers state that their...
containers have either a 10-year or an indefinite usable life. Our results show that rigid containers, even unused, had high levels of bacterial ingress. This study calls into question the assumption that rigid containers, regardless of duration of use, can maintain the sterility of their contents poststerilization. Performance validations for SPs using a static bioaerosol challenge do not account for the dynamic environmental events that occur in the health care setting, and thus a dynamic bioaerosol challenge should be incorporated into future standard validation methods. As increasing efforts are made to reduce SSIs, ensuring maintenance of poststerilization sterility should be a high priority.

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