Sterile Instrument Storage in an Austere Environment

Are Sterile Peel Packaging and Cellulose Wrapping Equivalent?

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ABSTRACT

Background: Recommendations for optimal temperature and humidity for sterile instrument storage vary according to different sources. Furthermore, there are limited data comparing methods of packing smaller, lightweight, low-profile instruments. The purpose of this study was to compare sterile peel packaging and sterile cellulose wrapping for sterile instrument storage in an austere environment characterized by elevated temperature and humidity. **Methods:** Stainless steel screws were sterilized and stored in either sterile peel packaging, sterile cellulose wrapping, or no packaging. Four groups were evaluated. Group 1 consisted of four screws in a sterile peel-pack envelope and served as a time-zero control. Group 2 consisted of two groups of five screws, each packaged with blue sterilization cellulose wrap. Group 3 consisted of two groups of five screws, each packaged in sterile peel-pack envelopes. Group 4 consisted of 10 non-sterile unpackaged screws, which served as controls. Screws from groups 2, 3, and 4 were then cultured for 6 and 12 weeks. Temperature and humidity values were recorded in the instrument storage area. **Results:** Average temperature was 21.3°C (SD 1.2°C; range 18.9°C–27.2°C) and average humidity was 51.7% (SD 3.9%; range 39%–70%). Groups 1 (time-zero control) and 2 (sterile cellulose wrapping) demonstrated no growth. After 6 and 12 weeks, groups 3 (sterile peel packaging) and 4 (control) demonstrated bacterial growth. **Conclusion:** The most common culture isolates were gram-positive rods and two common nosocomial *Staphylococcus* species. Sterile peel packaging was not found to be equivalent to sterile cellulose wrapping in austere environmental conditions.

KEYWORDS: *instrument sterility; austere environment; peel packing; cellulose wrapping; sterile instrument storage*

Introduction

Maintenance of instrument sterility during storage is critical for infection control in the operating room. Temperature and humidity are often closely monitored in areas where sterile instruments are stored.[1] Exceeding certain thresholds for temperature and humidity are believed to compromise sterile packaging of instruments.[2] However, recommendations for optimal temperature and humidity for sterile instrument storage vary according to different sources.[3–5] Similarly, recommendations for sterile packaging for smaller, lightweight, low-profile instruments lack consensus.[6]

To date, no direct comparison has been performed between methods of packaging sterile instruments in conditions where temperature and humidity deviate from recommended values. Therefore, the purpose of this study was two-fold. The primary aim was to evaluate sterile instrument storage in an austere environment with temperatures greater than 24°C and humidity greater than 60%. Secondarily, we sought to compare sterile peel packaging and sterile cellulose wrapping in sterilizing and storing smaller, lightweight, low-profile instruments. We hypothesized that sterilized instruments would have no bacterial growth after 12 weeks of storage in an austere environment characterized by high temperature and humidity. Furthermore, we hypothesized that there would be no difference in sterility maintenance between packaging methods.

Methods

This quality improvement study was conducted with command approval and was reviewed by the Public Affairs Office (PAO) and Judge Advocate General's Office (JAG) prior to being submitted for publication. The operating room was located in a remote, austere setting but had generator power, running water, and air conditioning units to assist with climate control. However, there were no dedicated air filters or laminar flow units. The operating room provided damage-control surgery capability for trauma patients. The air conditioning units were routinely turned off to deliberately raise the temperature in the operating room to assist with the resuscitation of trauma patients. Dust was commonly observed in the instrument storage area. However, consistent efforts to keep dust to a minimum and maintain the overall cleanliness of the instrument storage area were made.

Non-sterile stainless steel screws (#8 × 1" 304 Stainless Steel Phillips Modified Truss Head Wood Screws, BCP1211, BCP Fasteners, USA; https://bcpfasteners.com/) were used to represent small, lightweight, low-profile surgical instruments. Some examples of these instruments include forceps, scalpel handles, elevators, iris scissors, small self-retaining retractors, and smaller suction tips. Group 1 consisted of four screws in a sterile peel-pack envelope (Self Seal Sterilization Pouch

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3.5"×10", Model 8542028665, PlastCare USA, Chatsworth, CA, USA; https://plastcareusa.com/). Group 2 consisted of two groups of five screws, each packaged with blue sterilization cellulose wrap (Sterilization Wrap 30" × 30", Blue Autoclave Film, Single Layer Cellulose, B091BBFHDL, AMZ Medical, Libertyville, IL, USA; https://www.amzsupply.com/). Group 3 consisted of two groups of five screws, each packaged in sterile peel-pack envelopes. Finally, group 4 consisted of 10 nonsterile unpackaged screws, which served as controls.

All groups were sterilized by autoclave (Tuttnauer 2540M, Hauppague, New York, USA; https://tuttnauer.com/us) using the same method for routine sterilization of surgical instruments at 135°C for 25 minutes with a dry time of 30 minutes. Sterile indicators for peel-pack envelopes and sterile cellulose wrapping were utilized to ensure the desired conditions for autoclaving were reached (Verify Steam Indicator Tape 1", Steris Healthcare, Mentor, Ohio, USA; https://www.steris.com/healthcare; Comply SteriGage Steam Chemical Integrator 1243A, 3M, Saint Paul, Minnesota, USA; https://www.3m.com/). The screws in group 1 (time-zero control) were sent immediately after autoclaving for microbial culture to ensure initial sterility, and that sterility would not be compromised by transport to the microbiology laboratory. The screws from groups 2, 3, and 4 were then placed on a storage shelf in an operating room in an austere environment where temperature and humidity were monitored and recorded for 12 weeks. The temperature and humidity probe was placed on the same shelf as the instruments. Five screws from groups 2, 3, and 4 were sent for processing and microbial culture at 6 and 12 weeks.

The microbiology laboratory was located several hours away from the operating room facility in the U.S. Army Medical Research Directorate–Africa (USAMRD-A) lab based in the Center for Microbiology at the Kenya Medical Research Institute headquarters in Nairobi, Kenya, which is ISO 9001: 2015 certified. All packages were transported to the microbiology laboratory in non-sterile plastic wrap packaging and opened using strict aseptic precautions in a biosafety cabinet. The screws in each group were then transferred into test tubes containing thioglycolate broth (SIGMA Lot SLBX4246).[7] The media was quality checked to ensure the growth of aerobes and anaerobes using control strains *Staphylococcus aureus* (ATCC#29213), *Pseudomonas aeruginosa* (ATCC#27853), *Candida albicans* (ATCC#168053), and *Clostridioides difficile* (clinical isolate). To grow aerobic bacteria and fungi, screws were cultured in trypsin soy broth (OXOID Lot 2341585). The inoculated media were incubated at 35°C±2°C in ambient air for 14 days with daily monitoring for turbidity. Any tube with turbidity suggestive of growth was sub-cultured in duplicate sheep blood agar and MacConkey media plates, incubated under aerobic and anaerobic conditions, and then Gram stained. Anaerobic conditions were created using anaerobic jars with anaerobic sachets (Thermo Scientific Lot 9169LJ-4). A Biomerieux VITEK2 compact machine was available for the identification of gram-positive cocci (VITEK®2 GP ID), gram-negative bacteria (VITEK®2 GN ID), and fungi (VITEK®2 YST ID). VITEK2 cards were not available for gram-positive rods.

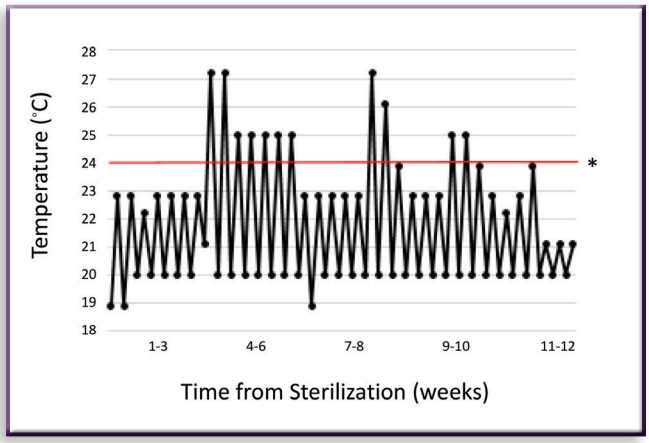
The degree of bacterial growth was graded by visual inspection daily and at the end of two weeks of incubation as the following: no growth (no turbidity), light (growth only on the top aerobic band), heavy (turbidity at the top and middle bands), or very heavy (turbidity throughout the whole tube).

Outcome measures were pooled, and standard deviations and ranges were calculated and reported for comparison. Microsoft Excel (version 2019) was used to collect and analyze outcome measures.

Results

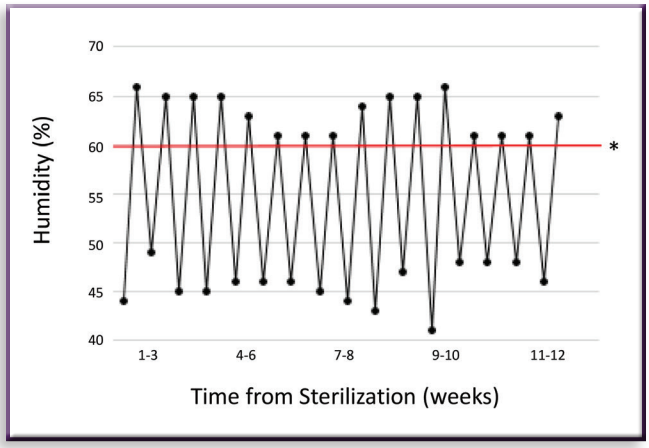
The average temperature in the instrument storage area was 21.3°C (SD 1.2°C) with a minimum temperature of 18.9°C and a maximum temperature of 27.2°C (Figure 1). The average humidity in the storage area was 51.7% (SD 3.9%), with a minimum humidity of 39% and a maximum humidity of 70% (Figure 2).

FIGURE 1 *Temperature values recorded over 12 weeks after initial sterilization.*



*represents 24°C temperature threshold for high temperature in study.

FIGURE 2 *Humidity values recorded over 12 weeks after initial sterilization.*



*represents 60% humidity threshold for high humidity in study.

Group 1 (time-zero control) demonstrated no growth. After 6 weeks of storage in an austere operating room, group 2 (sterile cellulose wrapping) demonstrated no evidence of growth. Groups 3 (sterile peel packaging) and 4 (control) demonstrated bacterial growth on day 1. Group 3 (sterile peel packaging) had very heavy bacterial growth, while group 4 (control) had heavy bacterial growth (Table 1).

After 12 weeks of storage in an austere operating room, group 2 demonstrated no evidence of growth. Groups 3 and 4 demonstrated bacterial growth on day 8 and day 1, respectively. Group 3 had moderate bacterial growth, and group 4 again had heavy bacterial growth (Table 2).

TABLE 1 *Characteristics of Microorganisms Isolated After 6 Weeks by Group*

Organism	Group
Aerobic gram-positive cocci*,†	3 (sterile peel packaging)
Aerobic gram-positive rods‡	4 (unsterilized control)

**Staphylococcus haemolyticus*.
†*Staphylococus epidermidis*.
‡Species not identified.

TABLE 2 *Characteristics of Microorganisms Isolated After 12 Weeks by Group*

Organism	Group
Aerobic gram-positive rods*	3 (sterile peel packaging), 4 (unsterilized control)
Anaerobic gram-positive rods*	4 (unsterilized control)

*Species not identified.

The isolates from the positive cultures were aerobic gram-positive cocci (*S. haemolyticus* and *S. epidermidis*) and unidentified aerobic and anaerobic gram-positive rods. No fungi were identified from the cultures.

Discussion

Higher temperature and humidity are believed to promote bacterial growth despite limited evidence from controlled studies. In addition, recommendations for optimal temperature and humidity vary according to different sources. This study provided data that support the hypothesis that higher temperature and humidity can promote bacterial growth in sterilely packaged and stored instruments. In addition, this study also found a difference in the maintenance of sterility when comparing two commonly used methods for sterile packaging. More specifically, bacterial growth was demonstrated when sterile peel packaging was used for sterile instrument storage for 6 and 12 weeks in a high-temperature and high-humidity environment. No growth was observed in any specimen sterilized and stored with cellulose wrapping. The most common organisms cultured were gram-positive rods and two species of *Staphylococcus*, which represent common nosocomial pathogens.[8]

Recommendations for temperature and humidity thresholds for sterile instrument storage vary according to different sources. The Association of Perioperative Registered Nurses has recommended temperatures ≤24°C and humidity ≤70%.[3] In contrast, the Joint Commission recommends a temperature range of 22°C–26°C and humidity <60%.[4] Other authors have recommended temperature ranges of 18°C–22°C and humidity between 35% and 50%.[5] By comparison, the present study selected temperature and humidity levels (>24°C and >60%) to define high temperature and humidity. Therefore, it is possible sterility compromise would occur at higher thresholds. However, previous work has not demonstrated this.

Bruna et al. compared two groups of surgical sterilized instruments stored at different temperature and humidity conditions following intentional contamination with *Serratia marcescens*.[1] The high-temperature and high-humidity group was stored at 35°C and 75% humidity, and the low-temperature and low-humidity group was stored at 20°C and 60% humidity.[1] No bacterial growth was detected in either group after 30 days of storage.[1] However, the authors only evaluated packaging techniques that wrapped sterile instruments. No evaluation was performed of sterile peel-pack envelopes—another common packaging method for sterilized instruments. Furthermore, sterility was evaluated after only short-term storage of 30 days.

Another study evaluated two methods of packaging sterilized screws stored in a clean operating room with no reported moisture or excessive dust.[8] No microbial growth was found after 96 weeks in the double-wrapped linen group or the sterile peel-pack envelope group.[8] Although the study evaluated two common methods used for packaging sterilized instruments, it did not evaluate the effects of high temperature and humidity thought to compromise instrument sterility.

The organisms cultured and isolated in this study are similar to those in the literature. Bhumisirikul et al. found *Bacillus* species, a gram-positive rod, and coagulase-negative Staphylococci were the most common organisms cultured in long-term storage of small surgical instruments in autoclaved packages.[9] These findings are similar to ours, which found gram-positive rods and coagulase-negative Staphylococci to be the most common culture isolates.

Overall mean temperature and humidity values in the present study fell below recommended thresholds. However, there were multiple instances where temperature and humidity values exceeded these thresholds (Figures 1 and 2). It is possible that exposing surgical instruments to varying temperatures and humidity led to instrument sterility compromise in the present study. Previous authors have shown fluctuations in temperature and humidity can lead to condensation, which can compromise sterile packaging.[10] In the present study, there were more days where humidity values exceeded thresholds than when temperatures exceeded thresholds (Figures 1 and 2). However, the contribution of excessive temperature or humidity to instrument sterility maintenance is unclear. Another possible explanation for the findings of the present study could have been differences between the layers of sterile packaging in the comparison groups. For example, the method used for wrapping the screws in group 2 (sterile cellulose wrapping) resulted in at least two layers of sterile cellulose wrapping. In contrast, group 3 (sterile peel packaging) had just a one-layer barrier. It has been suggested that using two layers of sterile packaging rather than one is more effective for instrument sterility maintenance.[11]

Another interesting finding of the present study was the lack of growth observed in group 1 (time-zero control). This suggests that while initial sterility can be achieved using single-layer sterile peel packaging, maintenance of sterility is limited in high-temperature and high-humidity environments. The time that lapsed from autoclave sterilization to microbial culture in group 1 was around 1.5 weeks. Therefore, it would be reasonable to suggest sterility compromise occurred sometime between 1.5 and 6 weeks in group 3 (sterile peel packing). Exploration of this occurrence in future studies would help to clarify these temporal differences.

In the present study, the timing and bacterial growth varied at 6 and 12 weeks in group 3 (sterile peel packing). At 6 weeks, group 3 demonstrated bacterial growth on day 1 that was characterized as very heavy. At 12 weeks, bacterial growth was demonstrated on day 8 and was characterized as moderate. There are multiple possible explanations for these findings. One possibility is unintentional contamination. This explanation could be supported by the fact that the bacteria cultured

at 6 and 12 weeks were different (Tables 1 and 2). In addition, the growth characteristics of the bacteria were also different. Another possibility is that transport conditions varied between groups sent for culture at 6 and 12 weeks. Continuous monitoring of temperature and humidity were not recorded during the transport of specimens. Therefore, exposure to more or less extreme conditions could have resulted in differences in bacterial growth. Lastly, the differences in bacterial growth could simply reflect differences observed in non-contaminated specimens despite exposure to different environmental variables. However, this explanation wouldn't account for the differences in the amount of bacterial growth. Nevertheless, the bacteria in the present study compare similarly to the organisms previously cultured in long-term storage of small surgical instruments in another study.[9]

Limitations

There are several limitations of this study. First, none of the gram-positive species were identified, yet they were the majority of the bacteria cultured. Antibiotic sensitivity of bacterial species was also not performed on isolated bacteria. While gram characterization and coagulase negativity provide useful information, specific resistance and susceptibility profiles would be more informative in understanding the clinical risk of the contaminating bacteria. Second, no spore load was used to verify that the autoclave reached appropriate conditions for the necessary amount of time. However, sterility indicators were used, and time-zero sterility was verified by sending a small sample of specimens for culture immediately after autoclaving (group 1), which were negative for microbial growth. Third, continuous monitoring of temperature and humidity was not recorded during the transport of specimens. Therefore, it is possible that conditions could have been more or less extreme than the storage conditions, leading to an under-appreciation of the actual conditions the specimens were exposed to. Fourth, study sample sizes were small and therefore limited robust data analysis, which may have altered the results. Lastly, this study used small stainless steel screws to represent small, lightweight, low-profile instruments. Thus, results for larger, more complex, and heavier instruments may differ.

Conclusion

In conclusion, bacterial growth was demonstrated when sterile peel packaging was used for sterile instrument storage in a high-temperature and high-humidity environment. No growth was observed in any specimen sterilized and stored with sterile cellulose wrapping. Gram-positive rods and two common nosocomial *Staphylococcus* species were cultured. Our study findings support using sterile cellulose wrapping over sterile peel packing for surgical instruments in austere environments. Future studies should consider evaluating the sterile storage of larger actual surgical instruments in high-temperature and high-humidity environments with continuous monitoring. In addition, culture sensitivities should also be performed for better microbial characterization.

Author Contributions

NL, DM, and LM conceived the study design. NL, DM, LM, and AW coordinated and collected the data. NL and CM analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Disclosures

The authors have nothing to disclose.

Funding

No funding was received for this work.

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PMID: 38412526; *DOI:* 10.55460/EB2S-XTB5

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JOURNAL *of* SPECIAL OPERATIONS MEDICINE™

Spring 2024
Volume 24, Edition 1

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