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Evaluation of the Sterility of Reynolds Wrap Aluminum Foil for Use During Rodent Surgery

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In biomedical research, surgeons are often responsible for simultaneously conducting rodent surgical procedures, monitoring anesthesia, and adjusting nonsterile equipment. Maintaining appropriate aseptic technique can be challenging when working under these conditions. Applying a sterile barrier material such as aluminum foil to nonsterile surfaces in these circumstances offers an innovative, inexpensive option to improve asepsis. The purpose of this study was to validate the sterility of food-grade aluminum foil for use as a sterile barrier on nonsterile equipment during rodent surgery. In this investigation, 10 boxes of aluminum foil were assessed for sterility by using ATP swabs and replicate organism detection and counting (RODAC) plates at 0, 14, and 28 d and 6 mo. At 6 mo, foil was applied to surgical equipment, and sterility was assessed by using ATP swabs and RODAC plates. Results revealed no ATP-positive results at any time point. During assessment of samples obtained directly from boxes, RODAC plates yielded minimal bacterial growth (1 cfu per plate) in 2 of the 10 boxes at initial testing and in 1 box at the day 0, day 14, and 6 mo time points. No growth was observed at day 28 (tested directly from the box) or at 6-mo apparatus testing. Our data revealed minimal bacterial growth on tested samples and support the use of Reynolds Wrap aluminum foil as a sterile barrier on nonsterile surfaces during aseptic rodent surgery.

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Strict adherence to correct aseptic technique is critical to ensure successful surgical outcomes. The *Guide for the Care and Use of Laboratory Animals* states that failure to maintain appropriate asepsis may result in physiologic and behavioral effects that negatively influence animal wellbeing and research outcomes.[10] Clinical and subclinical infections due to poor asepsis during rodent surgery can cause physiologic changes in parameters including fibrinogen levels, glucose concentrations, leukocyte counts, and histology, which can alter experimental results and delay postoperative recovery.[1,3,20,21] The use of appropriate aseptic techniques including correct preparation of the patient and surgical site, sterilization of surgical equipment, and the use of appropriate surgical techniques offer highly effective methods to reduce the likelihood of bacterial contamination.[19] Appropriate surgical practices include the use of sterile patient drapes and appropriate personal protective equipment and correct preparation of surgical equipment.

When considering rodent surgery in biomedical research, a single person (also known as the 'solo surgeon') typically is responsible for performing a surgical procedure while monitoring anesthetic depth and manipulating equipment including knobs of the anesthesia machine, handles on lights, microscopes, and parts of stereotaxic equipment. Maintaining sterile technique is challenging under these conditions. However, the application of a sterile barrier material to these nonsterile surfaces allows the surgeon to manipulate necessary equipment during the surgery using sterile gloves without compromising asepsis.

Several institutions have recommended various materials, including manufactured sterile plastic sleeves, commericial cling film, and aluminum foil, as sterile barriers on nonsterile surfaces during rodent surgery.[6,26,27] A recent study validated the sterility of cling film directly from the box as a sterile rodent drape,[6] but the sterility of food-grade aluminum foil has not been assessed until now. Aluminum foil is a disposable, durable, malleable material easily accessible worldwide for use as a sterile barrier in the rodent surgery setting. Although laboratory supply companies offer aluminum-foil products, these products can be relatively expensive as compared with aluminum foil sold for food preparation. The validation of food-grade aluminum foil as a sterile product gives researchers performing rodent surgeries an inexpensive, accessible material to enhance aseptic technique, comply with institutional policies, improve animal welfare, and increase the reliability of experimental results.

The purpose of the current study was to evaluate the sterility of a commonly available aluminum foil (Reynolds Wrap) by using ATP swabs and RODAC plates to validate the use of this material as a sterile barrier for nonsterile surfaces during rodent surgery. We hypothesized that aluminum foil would yield minimal bacterial growth for at least 6 mo.

Materials and Methods

Aluminum foil storage. Ten boxes of food-grade aluminum foil (75 ft^2, Reynolds Wrap aluminum foil, Reynolds Consumer Products, Lake Forest, IL) from several manufacturer lot numbers were tested during this study. To simulate storage under practical conditions, boxes were stored with the lid resting closed but unsealed on a shelf in the laboratory animal care program training room. Rodents were frequently handled under conventional conditions in this area for various instructional purposes. Ambient temperature and humidity were not recorded in this room. The exterior portion of the aluminum foil boxes was handled with non-gloved or nitrile-gloved hands; however, foil sheets were only manipulated or removed from

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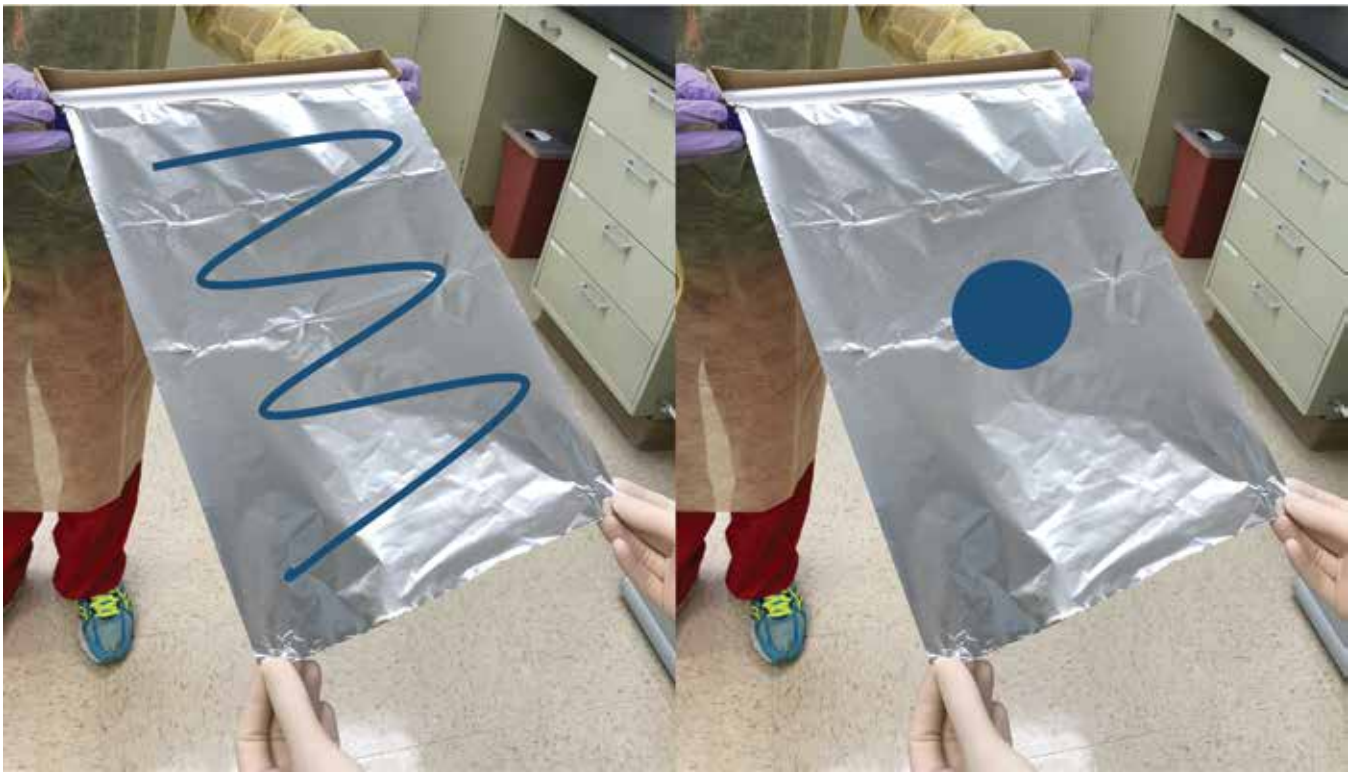

Figure 1. (A) The ATP swab was applied in a zigzag pattern to the front and back surfaces of the foil sampling area. (B) After ATP sampling, a RODAC plate was applied for 5 s to the front side of the foil in the center of the sampling area (blue dot). A second RODAC plate was then applied for 5 s to the center of the back side of the foil.

boxes using sterile gloves during testing and manipulation for this study.

ATP swabs. To detect organic material on test surfaces, ATP swabs (UltraSnap ATP Surface Test Swab, Hygenia, Camarillo, CA) and a luminometer (SystemSURE PLUS ATP Measurement System, Hygenia) were used as described previously.[6] Briefly, a luciferase assay was used for ATP bioluminescence detection, and results were reported in terms of RLU.[4] All ATP swabs were tested within 15 min of sample collection, according to manufacturer instructions.

RODAC plates. RODAC plates (Trypticase Soy Agar with Lecithin and Polysorbate 80, Benton Dickinson, Sparks, MD) were used to detect bacterial growth. After samples were collected, each plate was incubated for 72 h at 35 °C. At 24-, 48-, and 72-h time points, plates were removed from the incubator and colonies were counted. At 72 h, final colony counts were recorded for each plate. All observable colonies on experimental plates were submitted to the Ohio Department of Agriculture Animal Disease Diagnostic Laboratory (Reynoldsburg, OH) for aerobic culture and identification by using MALDI-TOF MS. Because bacterial growth was too numerous to count on all positive control plates, speciation was not performed.

Microbiologic testing and manipulation of aluminum foil. For each microbiologic testing time point, investigators donned a disposable gown, surgical face mask, and bouffant cap. The person who held the exterior of the aluminum-foil boxes wore nitrile gloves, whereas those who handled the aluminum foil itself wore sterile gloves (Confiderm LT Powder-Free Latex Surgical Gloves, McKesson, Irving, TX). Gloves were not changed throughout each testing interval, unless glove sterility was compromised.

An initial test using the first 30 cm of the roll was performed to assess foil sterility. The assistant wearing sterile gloves pulled the first 30 cm of foil from the roll, and an ATP swab was applied in a zigzag pattern along the front and back of the exposed section (Figure 1 A). Two RODAC plates were used per foil roll at each time point; one plate was applied for 5 s to the center of the shiny (front) side of the foil, and the other plate was applied for 5 s to the center of the matte (back) of the foil (Figure 1 B). At each successive time point (days 0, 14, and 28 and month 6), 30 cm of foil was removed from the roll and discarded; then a second 30-cm section of foil was removed from the roll aseptically for ATP swab and RODAC sampling. Care was taken to avoid contact between the foil and box exterior during sampling procedures. Positive-control samples for ATP swabs and RODAC plates were obtained from the exterior of the boxes.

To imitate the handling frequency for each box of aluminum foil, all boxes were manipulated twice weekly during the first 30 d of testing. For this procedure, 2 investigators each donned a disposable gown, surgical face mask, and bouffant cap. An assistant wore nitrile gloves, to hold the box open. By using sterile gloves, 60 cm of foil was removed from each box and discarded. Boxes were handled on days 1, 4, 8, 11, 15, 18, 22, and 25.

Microbial sampling of anesthesia and stereotaxic machines. After the 6-mo microbial testing, 5 boxes of foil were randomly selected, and pieces of foil from each box were applied to 5 anesthesia machines and one stereotaxic apparatus. Sterile gloves were used to remove and discard 30 cm of foil; then the lengths of foil needed to cover the selected apparatus surfaces were removed. For each anesthesia machine, the oxygen-flow knob and vaporizer dial were covered with foil (Figure 2 B and C). 30 min after foil application, an ATP swab was applied to all foil surfaces and then a RODAC plate was applied (5 s per application) to the flow knob and vaporizer dial at multiple

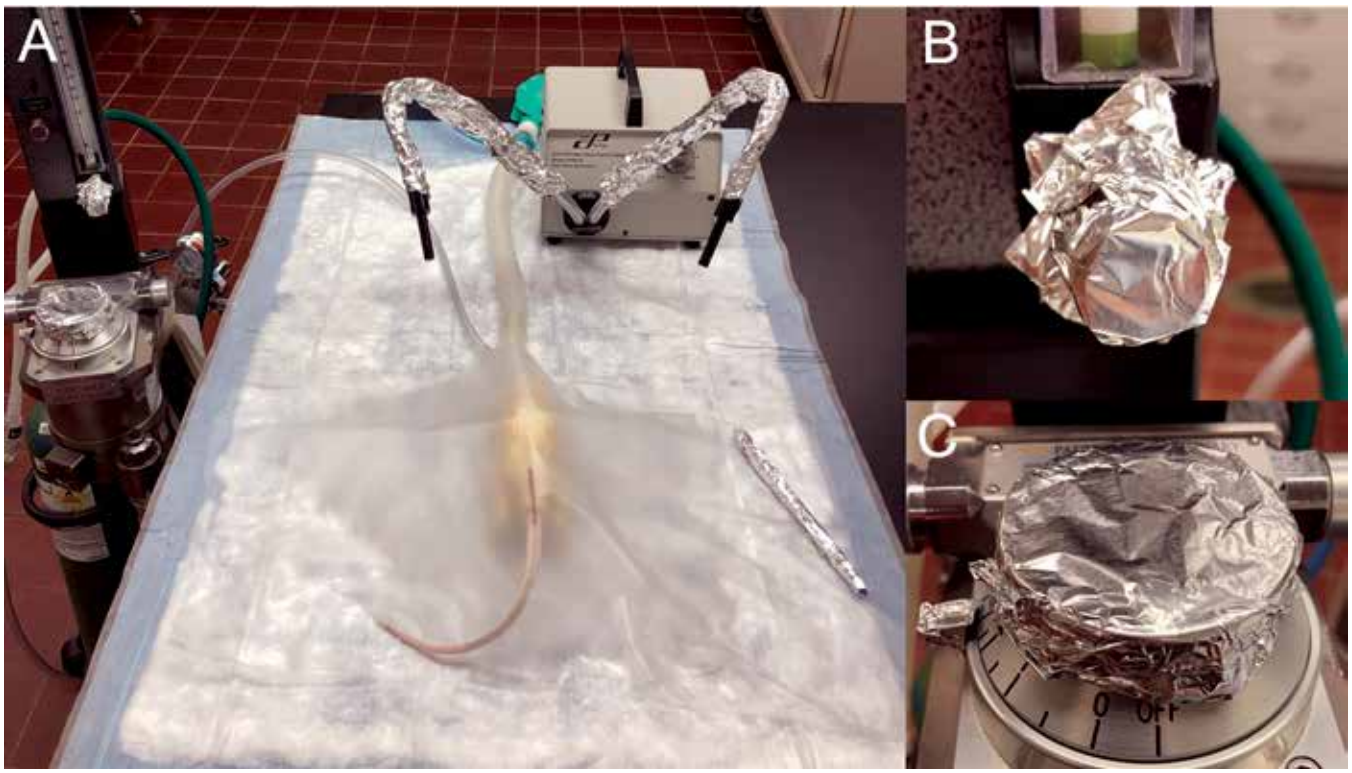

Figure 2. (A) Demonstration of a rodent surgical set-up using commercial cling film as a sterile drape over a Koken rat and aluminum foil applied to nonsterile surfaces including oxygen flow knob, vaporizer dial, light handle and writing implement, which has been placed on the cling film. (B) A magnified view of aluminum foil applied to the oxygen flow knob on the anesthesia machine. (C) A magnified view of aluminum foil applied to the vaporizer dial on the anesthesia machine.

angles to contact all covered surfaces (Figure 2). The same procedure was performed for the stereotaxic apparatus by using 4 selected knob locations (Figure 3). Positive-control samples for ATP swabs and RODAC plates were collected from uncovered surfaces of each piece of equipment.

Results

ATP analysis. Food-grade aluminum foil (n = 10) yielded 0 RLU on the initial opening of the box, on days 0, 14, and 28, and at the 6-month time points (Table 1). ATP swabs obtained from the foil-covered anesthesia machines (n = 5) and stereotaxic apparatus (n = 1) yielded 0 RLU (Table 2). Positive-control samples obtained from the exterior surfaces of the aluminum foil boxes and uncovered surgical equipment all had RLU counts that exceeded the institutional pass rate (>30 RLU).

RODAC plate analysis. A few samples yielded bacterial growth at the initial, day 0, day 14, and month 6 time points, but no bacterial growth was detected at day 28 or from samples collected from anesthesia or stereotaxic equipment at month 6 (Tables 1 and 2). At initial collection, the RODAC plate collected from the back of the foil yielded bacterial growth (*Bacillus oceanisediminus* and *B. infantis*, 1 cfu per plate) for 2 of the 10 boxes. At days 0 and 14, 1 of 10 boxes yielded 1 cfu per plate (*B. pumilus* and *B. circulans*, respectively). At 6 mo, 1 box yielded growth on 1 plate (*B. halosaccharovorans*, 1 cfu). All positive controls yielded more than 15 cfu per plate, which exceeded the institutional passing standards.

Discussion

The findings from our study validated the sterility of 10 boxes of Reynolds Wrap aluminum foil over the course of 6 mo. Our data show that ATP swab results did not exceed the institutional threshold at any time point. In addition, RODAC plates yielded minimal growth (1 cfu plate) from samples obtained from foil extended directly from 2 of the 10 boxes at initial testing and from 1 box at day 0, day 14, and month 6, with no growth at day 28 (tested directly from the box) or at the 6-mo apparatus testing. Thus, minimal bacterial growth was detected for at least 6 mo, supporting the use of Reynolds Wrap aluminum foil as a sterile barrier on nonsterile surfaces during rodent surgery in biomedical research.

All organisms identified in this study were from the *Bacillus* genus, which comprises Gram-positive, rod-shaped, spore-forming bacteria found throughout aquatic and terrestrial environments.[2,15,29] Most *Bacillus* spp. other than *B. anthracis* and *B. cereus* are considered to be clinically insignificant[24] and are frequently identified as culture contaminants.[7] *B. oceanisediminus* and *B. halosaccharovorans* have been isolated from high-salinity aquatic environments; pathogenicity has not been described for these organisms.[18,29] The *B. subtilis* group species (including *B. pumilus*) are widely used in industrial fermentation, agricultural, and pharmaceutical settings, given their enzymatic, antibiotic and probiotic capabilities.[2,11] *B. pumilus* is a component of several commercial human probiotic products, and mice have been used to assess the fundamental characteristics of these products.[5,14]

However, several *Bacillus* spp. can produce toxins that have been documented to cause illness occasionally in humans and animals. *B. pumilis* and *B. circulans* have been associated with rare cases of gastrointestinal and systemic disease in humans.[7,15,24] *B. circulans*-associated meningitis has been reported in humans predisposed to infection, such as patients with massive trauma, cancer, or immunodeficiency and neonatal patients.[15,28] The pathogenicity of *B. circulans* in animals has not yet been determined. One publication presented 4 cases of

Figure 3. A stereotaxic apparatus with aluminum foil applied to adjustment knobs. The blue arrows indicate the 4 knob locations sampled in this study.

Table 1. Results from ATP swabs (no. of RLU) and RODAC plates (cfu/plate) of samples from boxes (n = 10) of aluminum foil

	Initial	Day 0	Day 14	1 mo	6 mo
ATP swab	0	0	0	0	0
RODAC (foil front)	0	0	0	0	1[e]
RODAC (foil back)	2[a,b]	1[c]	1[d]	0	0

ATP swabs >30 RLU and RODAC plates with >15 CFU/plate exceeded institutional thresholds. All ATP swab and RODAC positive controls performed at each timepoint exceeded institutional thresholds.
[a]Organism identified as *Bacillus oceanisediminus*
[b]Organism identified as *Bacillus infantis*
[c]Organism identified as *Bacillus pumilus*
[d]Organism identified as *Bacillus circulans*
[e]Organism identified as *Bacillus halosaccharovorans*

B. pumilus-associated mastitis in dairy cows in Finland,[22] but we found no other reports of pathogenicity due to *B. pumilus* in animals. *B. infantis* was identified as one of 6 organisms cultured from a neonatal patient with sepsis, but the pathologic significance of *B. infantis* in that case was unclear.[13,17] Another group identified a strain of *B. infantis* with mimicry epitopes to cardiac myosin and the potential to induce myocarditis in A/J mice.[16,18] Although the evidence of potential pathogenicity of *B. infantis*, *B. pumilus* and *B. circulans* cannot be dismissed, these organisms are opportunistic pathogens and are ubiquitous in natural, household, and hospital settings.[8,15] Except for experimental inoculation of *B. infantis*, none of the *Bacillus* spp. cultured in our current study have been documented to cause natural disease in rodent species.

In total, 130 RODAC plate samples were collected from aluminum foil over the course of 6 mo, and 5 (3.8%) of those plates yielded minimal bacterial growth (1 cfu per plate). Given the ubiquity of the *Bacillus* genus, determining the source of these organisms is difficult. Colonization during the manufacturing process is likely, given that raw materials are extracted from the earth during the refining process.[25] We used the utmost care to handle all boxes aseptically; however possible contamination during collection cannot be excluded. We paid particular attention to removing the foil from the roll at a 45° angle, to prevent the back of the foil from contacting the cutting surface of the box and avoid contamination. Four of the 5 positive RODAC plates were cultured from the back (matte) side of the foil. Several studies have identified differences in the microscopic topography of thin aluminum-foil surfaces.[12,23] Scanning electron microscopy images consistently show a linear micropattern on the front side of the foil, which contrasts to a uniformly rough topography on the back surface. Although further investigation is needed, these differences in microscopic topography may play a role in the likelihood of organism adhesion to foil surfaces. We recommend using the front side of the foil as the contact surface for surgeons, thereby placing the back side of the foil in contact with the nonsterile surface. Similar to previous findings, our data indicate the highest microbial growth in the initial collection from sampled aluminum foil.[6] Therefore, we recommend discarding the first 30 cm of aluminum foil before each use to minimize chances of bacterial contamination.

At 6-mo apparatus testing, the positive-control RODAC plate collected from the stereotaxic equipment revealed no growth after 72 h of incubation even though results from ATP swabs exceeded the institutional threshold. The current study used stereotaxic equipment owned by the laboratory animal care program and designated for investigator training. We attributed the absence of bacterial growth to the relatively infrequent use of this equipment and thorough cleaning by skilled training staff after each use. While the use of a single stereotaxic apparatus was a limitation of this study, we successfully demonstrated the application of foil to high touch surfaces on this device for use in a practical setting.

Rodent surgeons have several material options available to facilitate appropriate rodent and equipment draping during surgical procedures. The adhesive nature and transparency of cling film provides an ideal material for a patient drape, and its sterility has been validated for at least 30 d.[6] At our institutions, we have noted that repeated contact of cling film with equipment surfaces can leave residues that may be detrimental for expensive or specialized equipment. However, this residue can be removed easily by using gauze soaked in isopropyl alcohol. In contrast, aluminum foil provides a residue-free option for application to nonsterile surfaces. Commercial plastic sleeves can provide residue-free barriers, but they also can be expensive and difficult to apply to specialized equipment surfaces without additional supplies to secure the plastic in place.[9] While we assume other food-grade aluminum products may be sterile, future studies are necessary to validate the sterility of other commercially available products and broaden the scope of options. Microbial sampling of foil-covered surfaces after rodent surgeries would be useful to establish standard intervals for reapplying foil to nonsterile surfaces.

If an assistant is not available to assist with foil application, the solo surgeon can dispense and apply this product independently. Prior to donning sterile garb, the surgeon can line the surgical table with cling film, taking care that the contaminated portion handled with nonsterile gloves falls over the edge of the tabletop. The surgeon can then dispense a portion of foil onto the surgical table, again taking care that the contaminated portion of the foil drapes over the edge of the table. Once the surgeon dons sterile garb, sterile scissors from the instrument pack can be used to cut the desired aluminum foil for application to nonsterile equipment.

Table 2. Results from ATP swabs and RODAC plates of samples from surgical equipment with and without application of aluminum foil

	Covered with foil		Control (no foil)	
Item	RODAC plate	ATP swab	RODAC plate	ATP swab
Nonrebreathing anesthesia machine no. 39	—	—	+	+
Nonrebreathing anesthesia machine no. 38	—	—	+	+
Nonrebreathing anesthesia machine no. 47	—	—	+	+
Nonrebreathing anesthesia machine no. 13	—	—	+	+
Rebreathing anesthesia machine no. 17	—	—	+	+
Stereotaxic apparatus	—	—	—	+

+ indicates an ATP swab with >30 RLU or a RODAC plate with >15 cfu.

Operative techniques, such as the use of sterile barriers on nonsterile surfaces, limit the risk of microbial contamination during surgery and are important tools for solo surgeons, who also must act as anesthetists and assistants during procedures. The validation of the sterility of Reynolds Wrap aluminum foil offers an innovative, inexpensive, and easily applied means to improve aseptic compliance and animal welfare in biomedical research. To our knowledge, such validation of the sterility of food-grade foil obtained directly from the box for use as a sterile barrier on nonsterile surfaces has not previously been published. Our data showed minimal bacterial growth on Reynolds Wrap aluminum foil for at least 6 mo after opening the box and support the use of this product during aseptic rodent surgery.

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