Attachment Potential and Survival of Bacterial Pathogens on Radiation Therapy Thermoplastic Immobilization Forms

Terrence J Ravine, PhD, MT(ASCP)
Patricia Brewer, MPA, R.T.(R)(T)
Sarah E Bru, MS, MT(ASCP)
Shannon Tyler, MD

Purpose To determine the attachment and growth potential of 4 bacterial pathogens linked to hospital-acquired infections to various thermoplastic immobilization forms.

Methods Four brands of heat-treated masks were inoculated with a known number of single bacterial species. Masks were sampled at 1-hour and 1-week time intervals. Bacterial colony counts were performed on these samples to determine initial attachment and survival capability. Ten radiation therapy clinics also were surveyed to determine current mask storage and usage conditions.

Results Only gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis* demonstrated an attachment capability at 1 hour, albeit greatly reduced (>99%) from the initial inoculum. Neither gram-negative *Escherichia coli* nor *Pseudomonas aeruginosa* demonstrated any attachment capability to the same mask surfaces. No bacteria were recovered from any mask at the 1-week sampling interval.

Discussion The considerable hydrophobic nature of the polycaprolactone material used in the construction of thermoplastic masks most likely prevents attachment of these bacteria when suspended in an aqueous (saline) solution. The addition of an antibacterial coating or incorporation of silver nanoparticles further reduces survival potential of these same bacteria. Preliminary results also indicate a substantial difference in gram-negative and gram-positive bacterial attachment capability.

Conclusion The tested bacteria do not readily attach to or survive long on the forms used in this study. The composition of the mask material, when combined with low humidity and room temperature storage, suggest a reduced risk to radiation therapy patients for acquiring an infection with these particular bacteria during intermediate to long-term therapy. However, it does not suggest a complete elimination of microbe transmission from improper handling of stored forms. Health care personnel must exercise care when applying these masks to prevent low-level patient contamination.

Keywords *thermoplastic immobilization, pathogen attachment, thermoplastic mask, bacteria, hospital-acquired infections*

More than a decade and a half since the report *To Err is Human*[1] brought patient safety to the forefront, there is still much work to be done to improve patient outcomes. Hospital-acquired infections (HAIs) have a significant effect on the morbidity and mortality of American patients, with a U.S. economic burden in the billions of dollars every year. At a given time, 1 out of every 25 people hospitalized in the United States is affected by 1 or more HAIs.[2] Some of the most prevalent HAI pathogens include methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus*, and *Clostridium difficile*.[3] These organisms, along with several others, are transmitted to patients from contaminated medical devices. The question addressed by this study is: "Can thermoplastic masks support long-term survival of bacteria associated with hospital-acquired infections?"

Literature Review

Thermoplastic immobilization devices routinely are used to position patients correctly during radiation therapy treatments. The devices are applied

repeatedly during a course of radiation therapy. Typically, this process involves several patient visits with some treatment courses lasting up to 6 weeks.[4,5] A previous study performed by the authors assessed the potential for microbial contamination of heating appliances (eg, water baths) used to warm these moldable plastic forms. Results suggested that these devices could serve as reservoirs for potentially infectious agents that pose a threat to radiation therapy patients.[6] The current study's objective is to expand upon this knowledge by evaluating the ability of different bacterial pathogens associated with HAIs to attach to or exist for up to 4 weeks on form surfaces. The potential risk of exogenous microorganism contamination from unprotected form storage also was addressed, but as a secondary objective.

Table 1

Test Microorganisms

Bacterium	Characteristics	Notes
Staphylococcus aureus (ATCC[a] 29213)	Gram-positive coccus, facultative anaerobe[b]	Methicillin resistant (MRSA)
Pseudomonas aeruginosa (ATCC 27853)	Gram-negative rod, obligate aerobe[c]	N/A
Enterococcus faecalis (ATCC 29212)	Gram-positive coccus, facultative anaerobe	Vancomycin susceptible
Escherichia coli (ATCC 25922)	Gram-negative rod facultative anaerobe	N/A

[a]*ATCC – American Type Culture Collection is a repository of microorganism strains.*

[b]*Facultative anaerobe – organism growth occurs with or without the presence of oxygen.*

[c]*Obligate aerobe – organism growth is oxygen dependent.*

For the study, we used uniform perforation head-only forms with incorporated frames acquired from CIVCO Medical Solutions, Klarity Medical Products, Orfit Industries, and Qfix. This specific form type was considered suitable for testing because of 2 main factors. First, its small size made for easy form handling and storage. Second, uniformly distributed perforations of similar character helped ensure consistency in microorganism application and subsequent mask sampling.

Test Organisms

Four microbes were chosen for an initial study (see **Table 1**). Selection was based primarily on their expected presence in health care environments and difficulty of their elimination. All 4 bacteria have been associated with HAIs and can survive for months on dry surfaces.[7] Several of these bacteria have the potential to become a larger problem because of increasing trends in antimicrobial resistance.

Collectively, tested bacteria represent 3 facultative anaerobes and 1 obligate aerobe that all can grow under the same incubation conditions (ambient air, 37°C, or 98.6°F). *C difficile,* an obligate anaerobe, was not chosen for this study because the pathogen would not be expected to survive in an aerobic environment where forms are stored. Two test microbes are categorized as gram-positive cocci and the other 2 are gram-negative rods. Gram-positive or gram-negative designations are determined by performing a Gram stain. This procedure is performed routinely in the clinical microbiology laboratory to help with microorganism identification. It is a 2-dye differential stain that separates bacteria by its resulting color into either a gram-positive (purple) or gram-negative (red) category. Gram stain reactions are based on differences in bacterial cell wall chemistry (see **Figure 1**). The terms cocci and rods refer to the morphology (shape) of the individual bacteria. Cocci are round in their overall appearance whereas rods are more elongated.

Each microbe was tested separately on 4 mask brands. Inoculated mask areas were sampled periodically for growth determination to establish initial bacterial attachment capability and length of time these 4 microbes could survive on form surfaces.

Form Composition and Uses

Both the CIVCO and Klarity masks (see **Table 2**) are composed of a polycaprolactone (PCL) base material (CIVCO, personal communications, November 2016; Klarity, personal communications, February

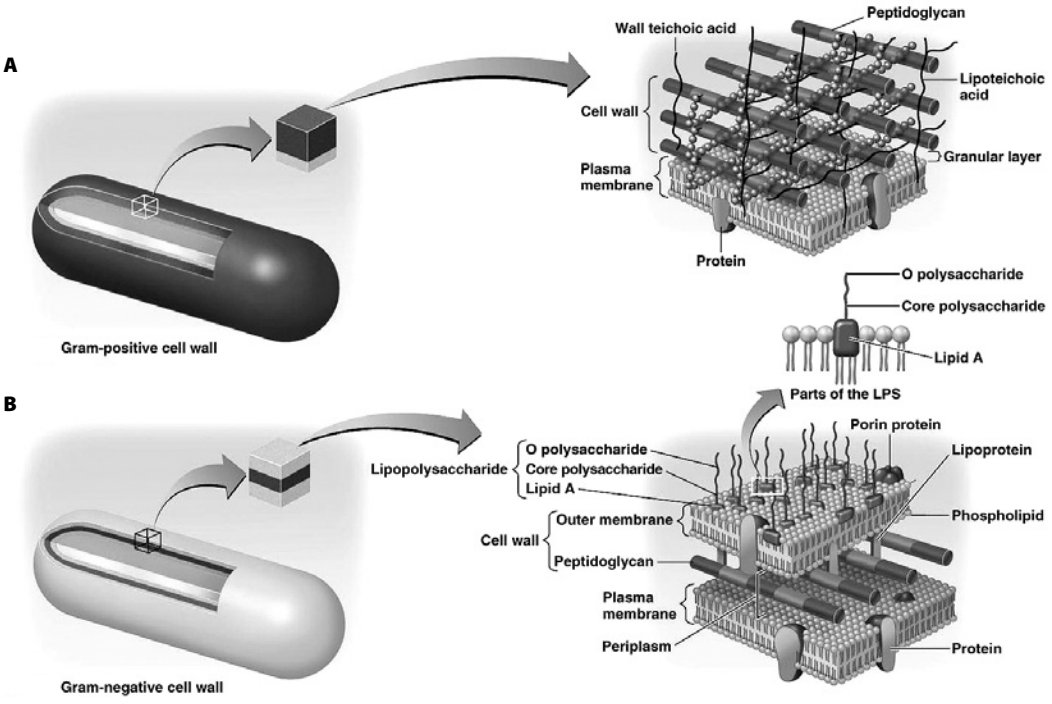

Figure 1. *Gram-positive and gram-negative bacteria. Difference exhibited by gram-positive (A) and gram-negative (B) bacterial cell walls. Abbreviation: LPS, Lipopolysaccharide. Images reprinted with permission from Tortora GJ, Funke BR, and Case CL.* Microbiology: an Introduction. *12th ed. San Francisco, CA: Pearson; 2016:82*

Table 2

Thermoplastic Masks for Testing

Manufacturer/Item	Description	Packaging (as received)	Appearance
CIVCO MTAPUD	Model: Uniframe type-S head Perforation: standard Thickness: 3.2 mm Material: polycaprolactone	Unwrapped	Opaque, shiny white surface
Klarity Medical R430UC	Model: EzeFrame head mask Perforation: 42% Thickness: 3.2 mm Material: polycaprolactone with a resin coating	Individually wrapped	Opaque, shiny white surface
Orfit Industries 35763/2MA/NH	Model: Efficast 3-point head mask Perforation: micro Thickness: 2 mm Material: polycaprolactone plus coating dispersion water based with antibacterial additive (per safety data sheet)	Individually wrapped	Opaque, matte (flat) yellow surface
Qfix/Aquaplast RT-1889	Model: Aquaplast RT U-Frame Perforation: standard Thickness: 3.2 mm Material: proprietary	Unwrapped	Opaque, shiny white surface

2017). Likewise, the Orfit mask is made of a PCL base material, as is indicated on the Orfit Safety Data Sheet.[8] The Qfix mask base material composition is unknown because the manufacturer considers the composition proprietary information (personal communication, November 2016). Based on appearance alone, the Qfix mask appears to be similar to those from CIVCO and Klarity. The Klarity mask has an additional proprietary resin coating and both sides of the Orfit mask contain antibacterial silver nanoparticles.[9] Silver nanoparticles often are incorporated into polymer matrices.[10] It is not known whether CIVCO, Klarity, or QFix have added an antibacterial substance to their masks.

Polycaprolactone is an exceptionally suitable material for making thermoplastic masks. This plastic type exhibits several favorable chemical and physical properties. It is a nylon polymer with a neutral charge, has a low melting temperature, is easily moldable, and can readily combine with other biological materials. PCL has a variety of medical uses, including encapsulated drug release, orthopedic pins and implants, wound dressings, contraceptive devices, degradable sutures, and dental sealants.[11]

Recent advances in tissue engineering demonstrate great promise for using PCL as a bioengineered skin substitute, in which an electrospun nanofibrous PCL membrane is created to serve as a support for tissue regeneration. The PCL meshwork functions as a substrate to attach naturally occurring extracellular matrix materials such as hyaluronic acid.[11,12] Furthermore, PCL with incorporated silver nanoparticles has been proposed for use as a wound dressing. The plastic-metal combination has been shown to inhibit growth of common wound pathogens substantially, including coagulase-negative *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* compared with PCL alone. The material also is regarded as a cost-effective method to reduce wound-associated infections.[13]

Incorporation of copper or silver into polymers occurs at incredibly low concentrations (ie, nanolevels) imparting biocidal activity to these materials. These 2 metals are good choices for adding to thermoplastics because of the metals' inherent stability under a variety of processing conditions.[10] A different formulation incorporates the antifungal agent ketoconazole in the core shell polymer matrix material of PCL-based magnetic hollow fibers. This blend has great potential for treating fungal infections.[14] Biologics such as chitosan, a substance derived from shellfish exoskeletons, also can be added to PCL to create a polymer with antimicrobial activity.[15]

Prior Evaluation

To the best of the authors' knowledge, no similar study has been performed. However, it should be noted that the Orfit Efficast mask had been subjected previously to antibacterial testing by the Japanese JIS Z 2801 method. This method determines the ability of an antimicrobial-treated surface to prevent microbial growth (bacteriostatic) or kill bacteria (bactericidal) after a contact period of 24 hours. The JIS Z 2801 method is referenced to the international industry standard ISO 22196:2011, measurement of antibacterial activity on plastics.[16] The JIS Z 2801 study demonstrated a 99.997% reduction in bacterial numbers.[8] The present investigation differed greatly from the Orfit evaluation in the bacterial strains tested, application/sampling process, storage conditions, and material treatment (ie, form heating).

The current study seeks to examine the capability of select bacterial pathogens to attach or exist for an extended period on supplied thermoplastic forms. Knowledge gained during this investigation will be used to determine the nature of the bacteria-thermoplastic relationship as it relates to patient infection risk.

Methods

Experimental Design

The study format was primarily quantitative in nature, as bacterial attachment and survival numbers were measured directly from periodic form sampling. The associated site survey was designed to provide supplemental information on form storage, handling, and cleaning conditions. A comparative design method involving 4 different masks was selected to reveal potential differences in microorganism-thermoplastic mask surface interactions. The study assumed that participating facilities could be using masks from several manufacturers for patient treatment. It also was

Table 3

Survey Checklist

Section Title	Items/Questions
Facility information	• Facility name • Facility identification number (blind) • Person answering questions • Date of survey • Survey method
Interview questions	• Average number of patients treated daily? • Are thermoplastic devices typically used daily in this department (yes or no)? • Thermoplastic brand used? • How are forms stored prior to use (original package, closed cabinet, or open shelving)? • How are forms stored after being formed for patient (closed cabinet or open shelving)? • Are gloves used when handling a patient's form (yes or no)? • Are forms shelved individually or stacked on top of each other due to limited space (individually or stacked)? • Are forms stored covered or uncovered? If covered, with what material? • Are forms cleaned between patient use (yes or no)? • If forms are cleaned, what cleaning solution is used (eg, soap and water, disinfectant)? • On a percentage basis, how many patients return for treatment within approximately 24 hours? • How are devices disposed of (regular trash or biohazard waste)?

expected that most clinical sites would demonstrate similar usage conditions, although some variation would be likely. This study was approved by the University of South Alabama Institutional Biosafety Committee (Protocol # 874097). It did not require local Institutional Review Board approval because no human subjects were involved.

Site Survey

A clinical site survey was developed and conducted to determine actual form storage and usage conditions. Parameters, such as storage room conditions, coverings, cleaning practices, handling methods, and others, were documented for each site (see **Table 3).** Ten radiation therapy facilities in 3 states agreed to participate in this survey. Surveyed sites were deemed representative of radiation therapy facilities in the region routinely using immobilization devices. Together, these 10 sites treat an average of 530 patients per day. Relevant site survey statistical data based on the average number of patient encounters per day includes:

- Range = 18 to 150
- Mean: 53
- Median: 45
- Mode: bimodal 30 and 45

Data Collection

A participant survey checklist was designed to collect site-specific information regarding thermoplastic mask usage. Information gathered during interviews with designated clinical representatives was documented on an Excel (Microsoft) spreadsheet for subsequent analysis. Similarly, laboratory experiment data log sheets also were designed to collect and document pertinent data (eg, recovered test microorganism numbers, room temperature and humidity conditions, and incubation times).

Stock Cultures

All bacterial cultures were acquired from the University of South Alabama Medical Center (USAMC) Microbiology Laboratory. They were maintained in refrigeration at 8°C (46.4°F) before testing. Stored bacteria were periodically transferred to fresh Becton-Dickenson R221239 5% sheep blood agar plates. All microorganisms were inoculated onto

a fresh 5% sheep blood agar plate and incubated overnight (18 to 24 hours) at 35°C (95°F) before test inoculum preparation. A 3-zone streaking method was employed to ensure that test microorganism cultures contained a single isolate. After testing, the investigators made a suspension of each microorganism in a freezing medium containing 20% glycerol in tryptic soy broth. These suspensions were stored at −70°C (−158°F) for long-term retention.

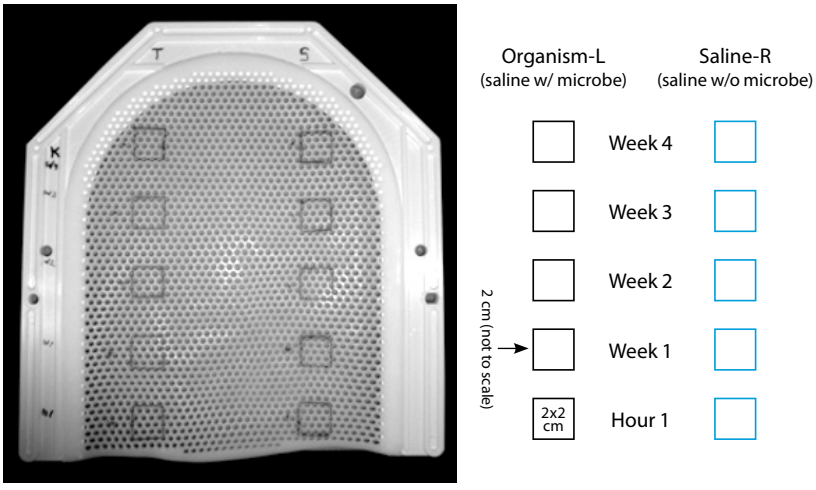

Figure 2. *Mask sample squares template. Thermoplastic mask with template-sketched 2 cm × 2 cm squares indicating inoculation areas on left. Radiographic film marking template layout with corresponding sampling areas indicated on right. Images courtesy of the authors.*

Sample Area Template

A sampling area template was prepared from a 10 in by 12 in sheet of Agfa Curix Ortho HT-G radiographic film (Agfa HealthCare NV). Overall template size was determined by applying the radiographic film to each test mask before cutting out test area squares. This step ensured that all sample areas would be located well inside of associated mask frames. Ten 2-cm by 2-cm (0.79 in × 0.79 in) squares, 5 on each side, were then cut out using an X-ACTO knife (Elmer's Products Inc).

Form Preparation

Each of the 4 test forms was individually heated in a COQ-1750W Adcraft convection oven (Admiral Craft) for 6 minutes at a temperature of ~74°C (165°F) until translucent. Additional mask support was provided by placing a 5-mm by 5-mm (~0.2 in × 0.2 in) wire grid on top of the oven shelf rack. This item prevented excessive mask sagging between the large wire shelf openings. This item was important for the thinner 2-mm thick Orfit mask. A piece of clean parchment paper also was placed between the mask and wire grid to prevent it from sticking to the grid. The Orfit mask required a slight modification so it would fit in the oven. The researchers used a pair of sterile surgical grade scissors to remove an approximately 4-in (10 cm) piece from the top of the form. Heated masks on parchment paper were transferred to a countertop cleaned with a suitable antimicrobial agent and allowed to cool for approximately 5 minutes until opaque.

While each mask was heating, the sample area template was wiped completely on both sides using a clean 5.08-cm by 5.08-cm (2-in × 2-in) gauze square (Fisher Scientific) saturated with 90% ethanol. The sample area template was thoroughly air dried before each use. It was subsequently applied to the cooled mask and 10 total squares (5 test and 5 control squares) measuring 2 cm by 2 cm (0.79 in × 0.79 in) were drawn (see **Figure 2**) on the patient contact side using a Medline DYNJSM02 sterile gentian violet marker.

Inoculum Preparation

A test microorganism suspension was prepared in a 25-mm by 120-mm (0.98 in × 4.72 in) sterile glass screwtop tube containing 4-mL sterile saline as follows. A sterile bacterial loop was used to transfer several well-isolated bacterial colonies from an 18- to 24-hour-old 5% sheep blood agar plate to the sterile tube. This step approximated a Remel 0.5% McFarland turbidity standard (Thermo Fisher). A 0.5% McFarland equivalence turbidity standard represents an approximate cell density of 1.5×10^8 colony forming units (CFU)/mL^{-1}.[18] Each bacterium was tested separately.

A negative control was prepared by transferring 4 mL of sterile saline to a separate glass tube. No bacteria

were added to this tube. This control functioned to indicate the sterility of the saline used in preparing test inoculums and determine whether exogenous contaminants were already present on masks as received.

A series of 10-fold serial dilutions (see **Figure 3**) were subsequently prepared in sterile 15-mL screwtop conical tubes from the sample inoculum. A 100-µL sample volume of the 10^{-5} to 10^{-7} dilutions were each transferred to a fresh 5% sheep blood agar plate and distributed evenly over the plate surface using a sterile cell spreader. All plates were then placed in a 35°C (95°F) ambient air incubator for an initial overnight incubation of 18 to 24 hours.

Form Inoculation

A sterile cotton-tipped swab was inserted into the prepared bacterial inoculum. Excess fluid was removed by wringing the wetted swab against the inner tube wall just above the saline solution before removing it. The swab then was rolled over the entire area of the first left-hand square while taking care not to go outside of the markings. Swab material was applied in 3 directions: vertically, horizontally, and obliquely at approximately 45°. The remaining 4 squares on the left side were similarly inoculated with test microorganisms using a new swab each time. The 5 right-side squares were treated similarly, but by using the sterile saline control without microorganisms.

Form Sampling and Storage

After 1 hour, the investigators sampled the lower-left test square of each form in the following manner: A sterile cotton swab was inserted into a 15-mL conical tube containing 1 mL of sterile saline. Excess saline again was removed by wringing the swab against the tube wall. The wetted swab then was applied to the target square and rolled again in 3 different directions, taking care to stay within the outlined borders. The swab then was returned to its tube and the sample stick was broken approximately one-half way up so that the swab tip remained in the capped tube. The right-side control square (saline only) also was sampled in a similar manner using a separate tube.

The investigators prepared a series of 10-fold serial dilutions from each sample as previously indicated. A

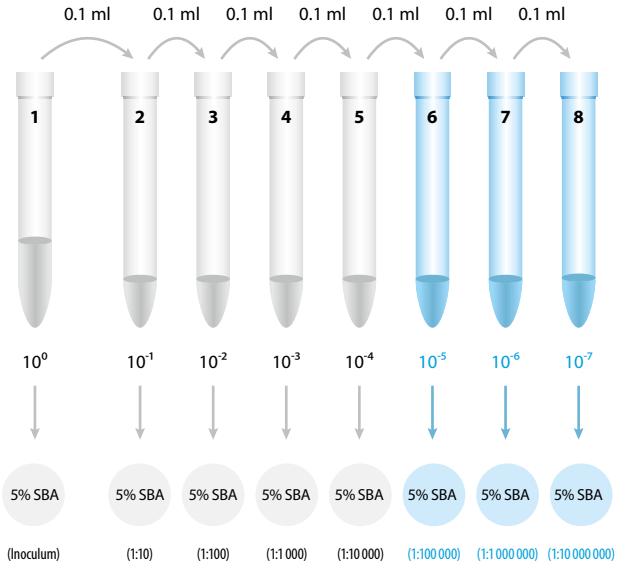

Figure 3. *Preparation of 10-fold serial dilutions of test microorganism to determine number of bacteria in initial inoculum swabbed onto mask surface (only dilutions shown in blue were made into spread plates for counting). Abbreviation: SBA, sheep blood agar. Image courtesy of the authors.*

100-µL aliquot of the 10^{0}, 10^{-1}, and 10^{-2} dilutions was transferred to a separate 5% sheep blood agar plate and distributed evenly over the plate surface using a new sterile cell spreader each time. The control sample was similarly processed but only a 10^{0} tube was transferred to a spread plate. Prepared plates subsequently were placed in a 35°C (95°F) ambient air incubator for an initial overnight incubation of 18 to 24 hours.

The investigators then transferred the sampled forms to an open-wire shelving unit while still resting on the parchment paper (see **Figure 4**). The shelving unit was positioned within 10 feet of the hallway door. This allowed air currents entering from the hallway to readily wash across form surfaces. The laboratory facility was kept secured and only testing personnel were able to access the facility. Each form was subsequently sampled after 1 week using this same method. Room temperature and humidity readings were periodically documented during the duration of each testing period.

Figure 4. *Mask storage area. Thermoplastic masks on wire rack representing open-shelving storage. A piece of parchment paper is seen underneath each mask. Image courtesy of the authors.*

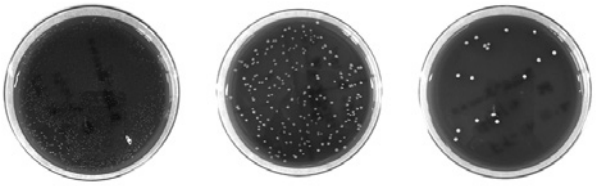

Figure 5. *Counting bacterial colonies. Direct plate counts showing, from left to right, the effect of increased dilutions with corresponding decreased amounts of* Enterococcus *colonies growing on 5% sheep blood agar. Each colony forming unit represents numerous rounds of bacterial division starting from a single bacterium. Image courtesy of the authors.*

Microorganism Enumeration

Dilution plates were removed from the incubator after an initial overnight incubation (18 to 24 hours) and examined for growth (see **Figure 5**). Colony counts were performed on dilution plates demonstrating 1 to 100 CFU. Resulting colony numbers were multiplied by the reciprocal of the tube dilution and then by 10 to account for the 100-µL sample size to determine CFU/mL^{-1}, as indicated in the calculation.

$$Colonies\ counted \times reciprocal\ of\ tube\ dilution \times 10 = total\ CFU/ml^{-1}$$

All plate counts were documented on constructed worksheets. Any plate demonstrating "no growth" was returned to the incubator for an additional overnight incubation before recording the plate negative for growth.

Results

Site Survey

Responses for the 10 surveyed sites are indicated in **Table 4.** The percentage of similar replies to each question also is indicated.

Table 4

Site Survey Responses (N = 10)

Questions	Reponses (%)
Form storage prior to use?	Closed cabinets (60) Open shelving (30) Packing box (10)
Form storage during use?	Open shelving (70) Closed cabinets (30)
Gloves used when handling forms?	No (70) Yes (30)
Stored individually or stacked?	Individually (100) Stacked (0)
Stored covered or uncovered?	Uncovered (100) Covered (0)
Cleaned between patients?	Yes, if open wounds/fluids present (90) No (10)
Cleaning agent used?	Sani-Cloths (50) Sani-Cloths or alcohol wipes (30) Alcohol wipes (10) None (10)
Disposal method?	Regular trash/given to patient (80) Regular trash (20)

One-hour Form Sampling

S aureus demonstrated limited attachment capability at the 1-hour sampling interval to only the Orfit and Qfix forms (see **Figure 6**). A similar result was not seen for either the Klarity or CIVCO forms, where no *S aureus* bacteria were recovered. In contrast, *E faecalis* demonstrated a greater attachment capability at 1 hour to all tested masks. Resulting plate counts

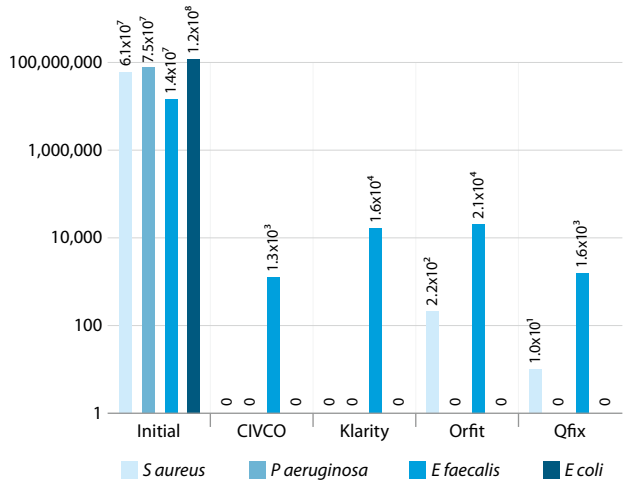

Figure 6. *Attached bacterial numbers. Organism recovery after initial 1-hour attachment period. Numeric labels above bars represent the number of CFU/mL^{-1} for each tested bacterium. Image courtesy of the authors.*

demonstrated that the attachment numbers for these 2 microbes were substantially reduced from the initial inoculum size (>99% reduction). Neither *P aeruginosa* nor *E coli* showed attachment capability to any form type for the same sampling period (See **Table 5**). No bacteria were recovered from the saline-only control side of any form.

$$\text{Percent reduction} = \frac{\text{No. Inoculated} - \text{No. Attached (1hr)} \times 100}{\text{No. Inoculated}}$$

One-week Form Sampling

In all cases, none of the tested microorganisms could be recovered from any mask at the 1-week testing interval. A similar result was noted for the corresponding saline controls. Consequently, testing was terminated when no bacteria could be recovered at the 1-week interval.

Discussion

The primary objective of determining several microorganisms' attachment/survival capability on 4 thermoplastic brands was completely fulfilled, and the secondary objective regarding potential of exogenous microorganism contamination from unprotected form storage was partially satisfied. The authors decided to terminate testing after each mask demonstrated negative results at 1 week. This decision was based primarily on the desire to conserve testing materials. Furthermore, 1 week was considered sufficient time for external contamination to occur.

Table 5

Percent Reduction of Inoculated vs Attached Microorganisms

	1-Hour Sampling (%)[a]			
Organism	CIVCO	Klarity	Orfit	Qfix
S aureus	100	100	99.99	99.99
P aeruginosa	100	100	100	100
E faecalis	99.91	99.88	99.99	99.85
E coli	100	100	100	100

[a] *100% = no attachment*

Microbe attachment to a surface is a prerequisite to its recovery. The ability of tested bacteria to attach to 4 different brands of thermoplastic forms was variable. Only *S aureus* and *E faecalis*, both gram-positive bacteria, demonstrated any attachment capability to mask surfaces, although at greatly reduced numbers (<1%) compared with the initial inoculum size (see Table 4). The difference seen in gram-positive and gram-negative organism results suggests a role for the cell wall in facilitating mask attachment (see Figure 1). It is not known which gram-positive cell wall component(s), if any, would contribute to this disparity.

Mask composition would be a further consideration in attempting to explain attachment differences (see Table 2). The chemical nature of a thermoplastic material imparts its physical properties. Accordingly, 2 relevant mask properties to evaluate would be material wettability and surface charge. Wettability is the capacity of a liquid to maintain contact with a solid surface. The nonwetting quality (hydrophobicity) of thermoplastic materials, such as PCL, might have contributed significantly to the negative *P aeruginosa* and *E coli* results, along with the low *S aureus* and *E faecalis* recovery at the 1-hour testing interval. The wettability of a surface is measured by water contact angle analysis. Contact-angle measurement determines whether a

material is more hydrophilic (tending to be wetted by liquid) or hydrophobic in its character. The higher the water contact angle, the more hydrophobic the material. Thermoplastic surfaces with contact angles greater than 90° are designated as hydrophobic. Water contact angles exceeding 150° are termed *superhydrophobic*. A native PCL contact angle of 129.97 has been recorded. This finding indicates that PCL exhibits great hydrophobicity.[19,20] Similar thermoplastic polymers, such as polyethylene terephthalate, polycarbonate, and polytetrafluoroethylene, also are highly hydrophobic.[21]

Material surface charge would be another factor influencing organism attachment. A positively or negatively charged thermoplastic surface could either help or hinder bacterial attachment. In this respect, synthetic polyester homopolymer PCL exhibits a neutral charge.[15] Therefore, this trait does not help to explain the differences seen in microbe attachment to the tested forms.

Upon removal from its shipping container, it was apparent that the Orfit mask visibly differed from the other 3 in general appearance. The Orfit device exhibited a yellow matte surface, whereas masks from CIVCO, Klarity, and Qfix appeared white and shiny (see Table 2). It was easier to draw test squares on the Orfit mask than to similarly mark the other 3 brands. In addition, only the Orfit mask test squares demonstrated wetness after inoculum application. This characteristic could be related to the antibacterial water-based surface coating. The recovery of 220 CFU/mL^{-1} of *S aureus* from the Orfit mask at 1 hour, along with the above observations, suggests that its surface is less hydrophobic than masks from the other 3 manufacturers. Incorporation of antibacterial silver nanoparticles into the Orfit mask might have decreased its overall hydrophobicity.[13]

At 21 000 CFU/mL^{-1}, the Orfit mask also demonstrated the greatest amount of *E faecalis* attachment of the 4 masks tested. This finding further implies a difference in mask hydrophobicity. The inability to recover a large number of these same 2 bacteria after 1 hour from the Orfit mask suggests 2 possibilities. First, the mask retained some of its hydrophobic quality. Second, incorporated silver nanoparticles were very effective at eliminating attached microorganisms within a short contact time. No explanation can be furnished for either the 10 CFU/mL^{-1} *S aureus* or 1600 CFU/mL^{-1} *E faecalis* results for the Qfix mask because its material composition is unknown (proprietary information). *E faecalis* attachment for the Klarity and CIVCO masks at the same 1-hour sampling interval was 16 000 CFU/mL^{-1} and 1300 CFU/mL^{-1}, respectively (see Figure 6). As previously indicated, *S aureus* could not be recovered after 1 hour from either of these masks. A negative 1-hour result for all 4 masks with *P aeruginosa* and *E coli* was unexpected. This finding could be related to differences in gram-negative cell wall components preventing attachment to mask surfaces.

S aureus, E faecalis, P aeruginosa, and *E coli* could not be recovered from any mask after 1 week. These results, combined with the low microorganism recovery at 1 hour, suggest that PCL mask hydrophobicity could have contributed substantially to a lack of bacterial attachment when applying bacteria suspended in an aqueous solution such as physiologic saline (0.89%). It is feasible that most (>99%) of the saline-suspended test organisms ran down inside the mask perforations toward the other side. If so, test organisms would have been found on the side opposite of patient contact. This possibility was not examined. A further contributing factor to negative growth results at 1 week of attached *S aureus* and *E faecalis* might have been the combination of low room humidity (average 33%) and room temperature (average 21.8°C, or 71.2°F).

The negative saline control result for all masks at both 1-hour and 1-week sampling periods suggests that relatively little risk of exogenous contamination to forms stored on an open shelving. There are 2 caveats: the study's storage conditions might not completely mimic those seen in a clinical radiation therapy setting, and the study's results only apply to the tested organisms. The results do not necessarily imply that the risk of mask contamination is likewise reduced for any other HAI organisms or even other brands of masks.

Concerning storage and microorganism contamination potential, all 10 surveyed treatment facilities stated that they did not stack masks. This was a desirable finding. Limited storage space should not negatively affect safe management of the masks. The practice of stacking similar masks (eg, head-only) on top of one another should be avoided because the

practice could pose a contamination risk. Our results suggest that formed (finished) masks can be stored safely uncovered when room relative humidity is low. Storage in a closed nonporous (nonbreathing) plastic bag can be considered but should be avoided in rooms in which high humidity is present. This statement is supported by Orfit literature specifying storage of finished masks in a dry place with a minimum temperature of 10°C (50°F) and maximum of 30°C (86°F). Further, Orfit literatures states that the humidity should not exceed 70%.[22]

Another potential issue is unintentional contamination of the mask by a health care worker while handling it without disposable gloves, a suitable protective barrier. Wearing gloves adds an extra layer of safety in preventing microorganism transmission. Accordingly, it is recommended that masks should be handled only by health care workers wearing clean gloves, which should be changed between handling of different patient masks.

The overwhelming majority (90%) of surveyed facilities reported cleaning masks if open wounds or fluids were present. Either Sani-Cloth germicidal disposable wipes (PDI Healthcare) or alcohol wipes were used for this purpose by 9 out of 10 facilities (90%). Care should be taken in cleaning soiled or contaminated masks so that any antibacterial coatings or incorporated elements are not altered or destroyed. For example, the Orfit mask literature specifies use of either soapy water or an isopropanol- or ethanol-based disinfectant. The use of "aerosol sprays, corrosive cleaning agents, solvents, or abrasive agents" is to be avoided.[22] Because ethanol is specifically mentioned, a methanol-based disinfectant might not be appropriate for use on this particular mask. In addition, a disinfectant containing benzyl ammonium chloride-quaternary ammonium chloride compounds, such as Sani-Cloth HB germicidal disposable wipes, might be a suitable cleaning agent, but individual mask manufacturers should be consulted before their use. The same can be said for alcohol-benzyl ammonium chloride-quaternary ammonium chloride containing Sani-Cloth AF3 wipes. However, therapists should probably avoid using a corrosive-containing Sani-Cloth bleach germicidal disposable wipe unless the individual mask manufacturer specifically approves its use. In all cases, therapists should consult the individual mask manufacturer's instructions before using any disinfectant.

The current study represents a limited evaluation of 4 HAI-associated pathogens. Further investigation is needed to evaluate additional factors. There are at least 3 possible avenues of future investigation. The first is performing a reproducibility study to confirm our present study findings. This would involve testing 3 masks from each manufacturer with the same 2 organisms (eg, *Enterococcus* and MRSA) that demonstrated 1-hour attachment. The second is to perform a study using shorter time durations such as 24 hours. The third would be to test different microbes to determine whether they pose a greater infection risk to radiation therapy patients. For example, opportunistic fungal pathogens such as *Candida albicans* and *Aspergillus fumigatus* might demonstrate a different attachment potential to these same forms than did the present study microorganisms. *Candida albicans* has been known to survive on inanimate surfaces for up to 4 months.[7] Moreover, *A fumigatus* spores might survive on mask surfaces for an extended amount of time. Actively growing *A fumigatus* is not always needed to cause a patient infection, as only the fungal spores can lead to disease. Moreover, these 2 fungal species can grow at 25°C (77°F),[23] a temperature quite near the average room temperature in the current study.

Likewise, it could be of interest to include a gram-positive bacterium from the *Bacillus* group (eg, *Bacillus cereus*). Like the fungus *A fumigatus,* the *B subtilis* bacterium forms spores. Although gram-positive in its nature, *Bacillus* spp. differs from *S aureus* and *E faecalis* in 2 respects. First, *Bacillus* is a rod-shaped bacterium, whereas *S aureus* and *E faecalis* are coccus-shaped. Second, most *Bacillus* spp. also can grow at room temperature, much like *C albicans* and *A fumigatus*. It should be recalled that a *Bacillus* spp. was one of the bacteria recovered from a heating appliance in the authors' previous study. This organism proved difficult to eliminate using routine cleaning methods, which was most likely due to the presence of residual spores. Finally, testing an obligate anaerobe such as *C difficile* also could prove relevant because *C difficile* forms spores. Although the vegetative (actively growing) form would not survive

ambient storage conditions, its spores have demonstrated the ability to do so for months at a time.[7]

Conclusion

Our results indicate the tested bacteria do not readily attach to the evaluated forms. Furthermore, those bacteria that initially attach do not survive for an extended time. The findings also imply that an open-shelf storage arrangement does not greatly increase the risk for external contamination. Wearing gloves during form handling is recommended and should be considered for inclusion in standard operating procedures. Collectively, these findings suggest that there is relatively little risk of test bacteria being transmitted to patients undergoing intermediate to long-term radiation therapy treatment using these particular thermoplastic forms.

This assertion is further supported by a 3-year review of pertinent University of South Alabama Medical Center infection control records indicating that no HAI related to thermoplastic mask use had been reported. This fact bodes well for patients who are susceptible to opportunistic bacterial infections. On a cautionary note, the current study only represents an initial attempt at determining the infection risk posed by thermoplastic form use. It in no way implies that there is zero transmission risk but rather a low risk, at least for the tested microorganisms and forms. Each radiation therapy facility should address their institution's storage and handling procedures to determine whether there is an opportunity to further reduce associated infection transmission risk.

Terrence Ravine, PhD, MT(ASCP), is an associate professor in biomedical sciences at the University of South Alabama in Mobile. He also is the Biosafety Officer for the Pat Capps Covey College of Allied Health Professions at the university. He can be reached at travine@southalabama.edu.

Patricia Brewer, MPA, R.T.(R)(T), is the radiation therapy program director at the University of South Alabama in Mobile. She can be reached at pbrewer@southalabama.edu.

Sarah E Bru, MS, MT(ASCP), is a retired instructor in biomedical sciences at the University of South Alabama in Mobile. She can be reached at sebru@southalabama.edu.

Shannon Tyler, MD, is an assistant professor of medicine in the division of infectious diseases, department of internal medicine at the University of South Alabama and the medical director of the department of physician assistant studies, Pat Capps Covey College of Allied Health Professions.

The authors would like to thank several people for their willingness to support this study. We thank the representatives of several companies for donating thermoplastic forms for testing, including Melissa Jensen, Sales Support Specialist, CIVCO Medical Solutions; Peter Larson, CEO of Klarity Medical; Marty Ratner, Vice President of Sales and Marketing for Orfit Industries; and Lisa Vandenberg, Senior Product Manager, Composites and Devices for Qfix. Special thanks to Peter Larson for loan of a convection oven and a monetary donation to help defray testing costs. We also thank the radiation therapy facilities participating in the study for their input and continued commitment to improving patient care.

Received March 28, 2017; accepted after revision on July 10, 2017.

Reprint requests may be mailed to the American Society of Radiologic Technologists, Publications Department, at 15000 Central Ave SE, Albuquerque, NM 87123-3909, or emailed to publications@asrt.org.

References

1. Kohn LT, Corrigan JM, Donaldson MS. *To err is human: building a safer health system*. Washington, DC: National Academies Press; 1999.
2. Magill SS, Edwards JR, Bamberg W, et al; Emerging Infections Program Healthcare-Associated Infections and Antimicrobial Use Prevalence Survey Team. Multistate point-prevalence survey of health care-associated infections. *N Engl J Med*. 2014;370(13):1198-1208. doi:10.1056/NEJMoa1306801.
3. Dancer SJ. Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. *Clin Microbiol Rev*. 2014;27(4):665-690. doi:10.1128/CMR.00020-14.
4. Uricchio N, Givens J, Keskemety J, et al. Computed tomography simulation procedures. In: Washington CM, Leaver DT, eds. *Principles and Practices of Radiation Therapy*. 4th ed. St Louis, MO: Elsevier; 2015:451-479.

5. Passmore GC. Overview of radiobiology. In: Washington CM, Leaver DT, eds. *Principles and Practices of Radiation Therapy*. 4th ed. St Louis, MO: Elsevier; 2015:58-86.
6. Brewer PS, Ravine TJ, Bru SE. Risk of patient infection from heating appliances used to produce thermoplastic immobilization devices. *Radiat Ther.* 2014;23(2):125-135.
7. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis*. 2006;6:130. doi:10.1186/1471-2334-6-130.
8. Efficast safety data sheet (version 1). Wijnegem, Belgium: Orfit Industries; 2010.
9. Orfit unique non-stick antibacterial coating technical information. (article 22096E). Wijnegem, Belgium: Orfit Industries; 2016.
10. Palza H. Antimicrobial polymers with metal nanoparticles. *Int J Mol Sci*. 2015;16(1):2099-2116. doi:10.3390/ijms16012099.
11. Woodruff MA, Hutmacher DM. The return of a forgotten polymer-polycaprolactone in the 21st century. *Prog Polym Sci*. 2010;35(10):1217-1256. doi:10.1016/j.progpolymsci.2010.04.002.
12. Figueira DR, Miguel SP, de Sá KD, Correia IJ. Production and characterization of polycaprolactone-hyaluronic acid /chitosan-zein electrospun bilayer nanofibrous membrane for tissue regeneration. *Int J Biol Macromol*. 2016;93(Pt A):1100-1110.
13. Thomas R, Soumya KR, Mathew J, Radhakrishnan EK. Electrospun polycaprolactone membrane incorporated with biosynthesized silver nanoparticles as effective wound dressing material. *Appl Biochem Biotechnol*. 2015;176(8):2213-2224. doi:10.1007/s12010-015-1709-9.
14. Wang B, Zheng H, Chang MW, Ahmad Z, Li JS. Hollow polycaprolactone composite fibers for controlled magnetic responsive antifungal drug release. *Colloids Surf B Biointerfaces*. 2016;145:757-767. doi:10.1016/j.colsurfb.2016.05.092.
15. Sahoo R, Jayshree SJP, Manirao R, Sahoo S. Blend effect of PCL on antimicrobial activity of different molecular weight chitosan. *Eur Sci J*. 2014;10(21):36-47.
16. ISO 22196. Plastics - measurement of antibacterial activity on plastics and other non-porous surfaces. 2nd ed. Geneva Switzerland: International Standard Organization; 2011.
18. McFarland equivalence turbidity standards (IFU 20410). Lenexa, KS: Remel; 2009.
19. Wasana K, Boonlom T, Paweena U, Pakkanun K, Wanida J. Surface modification of polycaprolactone scaffolds by plasma treatment for chondrocyte culture. *IPCBEE*. 2012;43.
20. Contact angle of water on smooth surfaces and wettability. KINO website. http://www.usakino.com/article/113.html. Accessed March 18, 2017.
21. Zilio C, Sola L, Damin F, Faggioni L, Chiari M. Universal hydrophilic coating of thermoplastic polymers currently used in microfluidics. *Biomed Microdevices*. 2014;16(1):107-114. doi:10.1007/s10544-013-9810-8.
22. Raycast high precision, efficast and pelvicast thermoplastic material for patient immobilisation instructions for use (31711USA version 7). Wijnegem, Belgium: Orfit Industries; 2017.
23. Antley PP, Hazen KC. Role of yeast cell growth temperature on Candida albicans virulence in mice. *Infect Immun*. 1988;56(11):2884-2890.