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Evaluation of a Flash Disinfection Process for Surface Decontamination of Gamma-irradiated Feed Packaging

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Ensuring biosecurity for pathogen-free rodents generally requires processing all materials that come in direct contact with the animals, including feed, to reduce or eliminate unwanted adventitious agents. A common method of processing animal feed is gamma irradiation. Irradiation is performed offsite and requires transport of feed from the irradiator to the point of use, potentially resulting in surface contamination of the packaging. We tested whether an autoclave could be used to provide a flash disinfection cycle to decontaminate the outer feed packaging while having a limited effect on nutritional feed quality. We developed a standardized and repeatable method, which involved attaching sterile glass vials containing *Pseudomonas aeruginosa*- and *Staphylococcus aureus*-laden culture broth onto the bag's surface, to validate effectiveness of the process. Nutritional analyses verified that the flash process had minimal effect on feed quality. Gas chromatography–mass spectrometry confirmed that subjecting feed packaging to the elevated cycle temperatures and pressures did not result in feed contamination by the packaging materials. The lowest autoclave setting that produced consistent surface disinfection, as determined by 3 consecutive negative cultures, was exposure of the bag surface to a chamber temperature of at least 82 °C for a minimum of 2 min. This flash disinfection process has been implemented successfully in 5 vivaria supporting more than 35,000 rodent cages daily.

Abbreviation: GC–MS, gas chromatography–mass spectrometry

Ensuring biosecurity for specific pathogen-free rodents generally requires that all materials, including feed, be processed prior to use in order to prevent introduction of unwanted adventitious agents. Historically, feed was steam-sterilized to destroy bacteria, viruses, and fungi.[11,18,19] Although highly effective when used appropriately, steam sterilization of feed leads to degradation of heat-labile nutritional ingredients, can increase pellet hardness, and (if feed is bagged) can result in pellet clumping.[6,9,10,14,16] Some packaging for autoclavable feed is perforated to allow steam penetration, but this characteristic increases the possibility of poststerilization contamination of the feed contents. Adamiker[1] demonstrated that irradiation could effectively replace heat and chemical sterilization methods as a means to reduce the bacterial and pathogen concentration of diets for germ-free and specific pathogen-free animals. The use of gamma-irradiated rodent feed was first described more than 25 y ago and was introduced commercially in 1984 as an alternative to steam sterilization.[12,13] Currently, irradiated rodent feed is used in many vivaria as a component of their institutional biosecurity programs.

After irradiation, feed must be transported from the irradiator to the point of use, potentially resulting in surface contamination of the packaging. Conventional methods developed to address this risk involve aseptically removing the outer paper packaging from the underlying plastic liner, spraying the bag with an appropriate disinfectant, or passing the plastic liner or plastic bag-encased feed through a disinfectant dunk tank. However, these methods are labor-intensive or are subject to personnel performance. We hypothesized that subjecting feed bags to a brief flash disinfection cycle in a steam sterilizer could be used to decontaminate the outer packaging while having a limited effect on nutritional quality and pellet hardness. Flash sterilization techniques are used to rapidly resterilize instruments or fragile devices that cannot undergo prolonged sterilization procedures.[5]

We developed a method to decontaminate the surfaces of feed bags rapidly and efficiently while minimally affecting feed characteristics. In addition, we devised a standardized and repeatable technique, involving both *Pseudomonas aeruginosa* and *Staphylococcus aureus*, to validate the effectiveness of the process. Nutritional analyses were performed to verify that the process had minimal effect on feed quality, and gas chromatography–mass spectrometry (GC–MS) was used to verify that subjecting the feed packaging to elevated temperature does not result in the release of packaging constituents, especially those in the inner plastic bag, resulting in feed contamination.

Materials and Methods

Autoclave cycle development and testing. Initial autoclave cycles were developed using a pass-through prevacuum sterilizer (model B301850-462, Amsco Scientific Eagle Century Series, Steris Corporation, Mentor, OH). Testing was conducted using single bags of irradiated feed (Lab Diet 5053, PMI Nutrition International, Brentwood, MO), which were placed on a shelf in the center of the autoclave rack. An extremely short flash cycle was developed with the intended goal of destroying all vegetative bacteria on the bag's surface. The cycle was developed by programming a gravity cycle at a sterilization temperature of

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100 °C, the lowest programmable temperature setting possible on this and most commercial autoclaves, with different sterilization times depending on bacterial culture results, which were obtained immediately after opening the autoclave. From a total cycle length of 330 s, cycle times were reduced by 30 s after each consecutive negative culture result until a positive culture was obtained (at 120 s). The shortest cycle parameters that consistently yielded 3 negative bacterial culture results was a gravity cycle of 150 s at a sterilization temperature of 100 °C for 0 s. This cycle achieved a maximum chamber pressure of 28.7 lb/in.^{2}. With these parameters, after reaching the set temperature, the autoclave would progress directly into the exhaust phase, exhausting to 4.0 lb/in.^{2} as the chamber temperature decreased to 92.7 °C. During the subsequent vacuum dry phase, internal chamber pressure fell to 0 lb/in.^{2}. The cycle ended with an air break, allowing the machine to be safely opened and the feed removed.

Given these initial results and the known combined effects of time and temperature in the destruction of vegetative bacteria,[17] we sought to use a lower temperature (82 °C) in a gravity autoclave cycle for decontamination of feed packaging surfaces. We selected this temperature because it is the traditional and effective temperature used for sanitation of inanimate surfaces in the context of mechanical washing equipment, such as cabinet and tunnel washers. A pass-through prevacuum sterilizer (model 5596EP-1V, Tuttnauer, Hauppauge, NY) with customized software, created with the assistance of the autoclave's manufacturer, was used to provide a reduced-temperature cycle. The customized software enabled us to create a flash gravity cycle with the following settings, ranges, run times, and cycle phases: no prevacuum phase, a sterilization temperature range of 82 to 100 °C, a sterilization time from 0 to 10 min, a dry phase range of 0 to 10 min, and a brief exhaust phase. The drain located at the bottom of the chamber remained open during the cycle, preventing steam pressure from accumulating within the chamber. In addition to using a lower sterilization temperature, we sought to reduce the potential time during which the feed was subjected to an elevated temperature. The software activated the automated door on the unload side of the autoclave to open at the conclusion of the cycle to further disseminate heat from the chamber.

The peak chamber temperature of the shortest cycle that consistently yielded 3 negative bacterial culture results was determined for both autoclaves. A thermocouple digital thermometer (model 52 II, Fluke, Everett, WA) was used for measurement. The probe thermocouple was placed in the center of an empty chamber, and peak chamber temperature was determined during each of 3 cycles in each autoclave.

Confirmation of decontamination of packaging surface. Bacterial cultures were prepared by the Laboratory of Comparative Pathology (Research Animal Resource Center, Memorial Sloan-Kettering Cancer Center, New York, NY and the Weill Medical College of Cornell University, New York, NY). *Pseudomonas aeruginosa* (accession number, 27253; American Type Culture Collection, Rockville, MD) and *Staphylococcus aureus* (accession number, 29813, American Type Culture Collection) bacteria were maintained on standard sheep blood agar (BBL TSA II 5% SB, Becton Dickinson, Sparks, MD) in a 37 °C, 5% humidified incubator (model NU8500, IR Auto Flow Water Jacket Incubator, Nuaire, Plymouth, MN). At 24 h prior to use, culture tube vials (model 60818-496, VWR Scientific Products, West Chester, PA) of both bacteria were prepared that contained a 0.5-McFarland suspension in 5 ml of 0.85% (w/v) normal saline solution. The suspension turbidity was standardized at 105 CFU/ml by using a colorimeter (model 52-1210, Vitex Special Colorimeter DR 100, Hach, Loveland, CO). We used 4 variations of validation methodology: 1) direct inoculation of culture broth onto the bag's surface; 2) attachment of sealed paper envelopes containing bacteria-laden filter paper strips onto the bag's surface; 3) attachment of sterile glass culture vials each containing a bacteria-laden filter strip onto the bag's surface; and 4) attachment of sterile glass vials containing bacteria laden culture broth onto the bag's surface.

For direct inoculation, 2.5 × 10-cm rectangles were drawn (Sharpie Permanent Marker, Stanford, Bellwood, IL) on the upper surface of the bag and labeled appropriately. By using a sterile swab (Bacti-Swab NPG, Remel, Lenexa, KS), broth culture of each bacterium was inoculated directly onto the bag's surface by liberal wiping of the designated area with a broth-moistened swab. After application, each swab was evaluated by aerobic bacterial culture. In addition, sterile swabs were used to culture each inoculation site—immediately before and after flash disinfection, swabs were wiped over the inoculated surface and then submitted for aerobic bacterial culture.

For the techniques using bacteria-impregnated strips, 2 sterile filter-paper strips (1 × 5 mm; model 28310-140, VWR Scientific Products, West Chester, PA) were inoculated into vials of *P. aeruginosa* and *S. aureus* suspension cultures and incubated at 37 °C for 2 h. After incubation, the strips were removed with sterile forceps, and each strip was placed in a sterile, empty culture vial. The strips were dried by incubating the strip containing vials at 37 °C for 24 h. Shortly before use, 1 each of the dried strips incubated with *P. aeruginosa* or *S. aureus* was transferred into a regular unsealed mailing envelope (3 7/8 in. × 8 7/8 in.), and the remaining strip was retained in the culture vial as a control. The strip-containing envelopes were taped (Comply Indicator Tape [Steam], 3M, St Paul, MN) to the surface of the feed bag prior to flash disinfection. After the bag completed flash disinfection, the envelopes were collected, and the control and test strips were submitted for aerobic bacterial culture. Alternatively, strips prepared as described were placed in sterile empty culture vials (12 × 75 mm; model 60818-496, Culture Tubes with Closures, VWR Scientific Products), which were taped to the surface of the feed bag prior to autoclaving. Immediately after autoclaving, vials were retrieved and the strips collected and cultured.

For the techniques using culture vials of inoculated broth, culture vials (BD BBL Prepared Culture Media, Becton Dickinson) were inoculated 24 h prior to use with a 0.5-McFarland bacterial suspension in 5 ml of 0.85% (w/v in normal saline solution) and incubated. Suspension turbidity was standardized at 105 CFU/ml by using a colorimeter. Broth-containing vials of each bacterium were taped to the surfaces of feed bags prior to autoclaving.

GC–MS analysis of chemical contamination. To ensure that the disinfection process did not adversely affect the feed contained within the multi-ply packaging (consisting of a 3-layer paper bag and an inner plastic liner of 70% zero-slip low-density polyethylene fractional melt and 30% high-slip butene linear low-density polyethylene), feed was analyzed for chemical contamination due to package heating. Before and after flash disinfection, the plastic liner and feed were extracted with dichloromethane and analyzed by GC–MS (model 5989A mass spectrometer equipped with a model 5890 Series II gas chromatograph, Hewlett Packard, Palo Alto, CA).

Nutritional analysis. Feed samples were analyzed to determine whether flash disinfection degraded heat-labile feed constituents. Nutritional analysis was conducted by a commercial

laboratory (NP Analytical Laboratories, Checkerboard Square, St Louis, MO). The analytical methodologies used were those published by the Association of Official Analytical Chemists International.[3,4] The following samples were evaluated: a control sample of irradiated feed not subjected to flash disinfection; feed collected immediately after completion of the flash cycle; and feed collected after remaining in the autoclave chamber (with the door closed) for 1 h after completion of the flash cycle. The laboratory was blind to sample type.

Results

Development and testing of autoclave cycle parameters. The shortest autoclave cycle that resulted in consistent surface decontamination of rodent feed bags, as determined by 3 consecutive negative cultures, had a display temperature of 82 °C for a minimum of 2 min in a prevacuum autoclave using a gravity cycle and customized software. The peak chamber temperature during this cycle was less than 5 °C greater than the display temperature. Consistently negative cultures also were obtained with the prevacuum autoclave by using a gravity cycle and standard software, but the lowest possible display temperature was 100 °C, and the peak chamber temperature reached 141 °C.

Verification of decontamination of packaging surface. The 4 methods used to confirm bacterial kill during a gravity cycle were: 1) direct inoculation of culture broth onto the packaging surface; 2) attachment of sealed paper envelopes containing bacteria-laden filter paper strips onto packaging; 3) attachment of vials containing bacteria-laden filter strips onto packaging; and 4) attachment of vials containing bacteria-laden culture broth onto packaging. All 4 methods confirmed cycle effectiveness according to the criteria we established. However, several of the strip-containing envelopes tore due to dampness after autoclaving, and the plastic closures used on the vials were heat-sensitive and melted.

Nutritional analysis. Three samples—nonautoclaved irradiated feed (control), feed collected immediately after flash disinfection, and feed collected 1 h after completion of flash disinfection—underwent nutritional analysis. The testing laboratory concluded that there were no significant differences in composition among the 3 samples, all of which were similar to the published guaranteed analysis for the diet.

GC–MS analysis of chemical contamination. GC–MS analysis of the plastic liner of the packaging yielded 2 low abundance peaks, which could not be identified on the basis of a match to a compound in the reference library. These peaks were not present in any of the feed samples analyzed by GC–MS. Extraction and analysis of feed pellets obtained before and after disinfection yielded peaks that matched known ingredients or that failed to match any compound in the reference library. Both pre- and postdisinfection samples had the same matchless peaks, although differences in abundance were present.

Discussion

The movement of feed into a vivarium poses the risk of introducing unwanted adventitious agents. Precautions to protect against this risk are frequently labor-intensive and dependent on staff performance. To minimize the possibility of human error during the movement of large volumes of feed into a vivarium, we evaluated whether moist heat, provided by a steam sterilizer, could efficiently decontaminate the outer, paper surface of irradiated rodent feed bags without adversely affecting the bag's contents.

The effect of heat on the nutritional quality of rodent feed is well recognized.[7,9,10] We evaluated several flash disinfection cycles, with the goal of obtaining a brief, low-temperature cycle to reduce the possibility of deleterious effects on the feed. In addition, we were concerned that heat would release plasticizers or other detrimental constituents from the plastic liner or other packaging components to contaminate feed pellets (especially those in direct contact with the plastic liner), because the packaging was not designed for heat sterilization. However, none of the evaluation methods used revealed either nutritional degradation or chemical contamination of feed postdisinfection.

In late 2005, after we conducted the described studies, the feed manufacturer changed the packaging material used for irradiated diets. The liner was changed to a more durable high-density polyethylene plastic to prevent the possibility of microscopic pinholes, which could lead to contamination of diet after irradiation. To confirm that flash disinfection of the new packaging material did not adversely affect the feed contained inside, a contract laboratory (using the previously described methods) repeated the procedure for testing feed for chemical contamination. No chemical contamination was detected.[8]

We selected 2 bacterial species to serve as surrogates for many, but not all, of the adventitious agents that might contaminate the surface of feed packaging. Cultures of the gram-negative bacterium *P. aeruginosa* and the gram-positive bacterium *S. aureus* were either inoculated directly onto the surface of the feed bag or placed in various containers and attached to the bag's surface. After autoclaving, samples and appropriate controls were collected and submitted for aerobic culture.

Various problems were encountered with several of the validation methods we used. The dampened paper envelopes frequently tore postdisinfection, resulting in contamination of the test strips inside. The tops of filter-containing glass culture vials frequently melted during heating. However, concerns regarding the routine handling of live bacterial cultures by personnel with limited experience in microbiologic technique limited the use of albeit effective direct-bag inoculation to experimental use rather than as a routine validation method. We considered the use of broth-containing culture vials with heat-resistant screw caps to be the most effective and safe routine validation method. These vials do not need to be opened outside of the laboratory in which they are prepared, broth-containing vials can be prepared in advance, and the cultures remain viable under refrigeration for as long as 3 mo.[2] Currently, this method is used monthly in each autoclave as part of our quality assurance program for the disinfection process.

When evaluating the chamber conditions using the bulk autoclave with standard operating software, we determined that this cycle resulted in exposure of the feed bag to temperatures of at least 82 °C for 2 min. Given the relationship between temperature and time of exposure on vegetative bacteria, we hypothesized that a program that limited the peak chamber temperature to approximately 82 °C and held that temperature for 2 min would achieve the desired bacterial kill while reducing the likelihood of nutritional degradation from heat[15] and of contamination of feed from packaging. Testing using the autoclave with the customized program confirmed our speculation.

In most bulk autoclaves, the temperature sensor is located in the drain at the bottom center of the vessel's chamber. Because the peak temperature inside the chamber may exceed that measured at the drain, the maximal temperature to which the surface of the feed bags is exposed may be greater than the set sterilization temperature of the cycle used. We observed this result in the autoclave operating with standard software—the

maximal temperature to which the bag's surface could be exposed was as high as 141 °C. However, we did not detect contamination of feed from the plastic liner or nutritional degradation of feed even at that temperature. In the autoclave operating the customized software, the chamber drain remained open during the entire cycle, thus limiting the increase in the peak chamber temperature to less than 5 °C greater than the display temperature.

The maximal number of feed bags that can be flash-disinfected per cycle varies depending on the autoclave's chamber size and the racking system available—the bags cannot be stacked or touching. We have successfully implemented and validated this method for processing rodent feed in 5 bulk sterilizers, which support a daily mouse population of more than 35,000 cages in 5 vivaria. The largest sterilizer can process 32 bags (25 lb each) of rodent feed per cycle. In conclusion, we have found flash disinfection of rodent feed to be a highly effective and efficient method to use as one component of our rodent biosecurity program.

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