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Test to Determine Sterile Integrity of Wrapped Medical Products at a Probability of Recontamination of 1 : 1,000,000

Hartmut Dunkelberg, MD; Susanne Rohmann, MS

OBJECTIVE. We developed a microbiological test to detect the penetration of airborne microorganisms through the packaging of medical products after sterilization, to meet the requirements of European standard EN 556. We applied this test method to transparent pouches.

DESIGN. The microbial-barrier properties of the transparent pouches were determined using the microbial challenge test, in which the package was placed inside an exposure chamber and exposed to a defined aerosol of *Saccharomyces cerevisiae*. The atmospheric pressure in the chamber was periodically reduced by 0-75 millibars, to simulate weather-dependent pressure changes. Thermoresistant petri dishes filled with nutrient agar were integrated into the transparent pouches before sterilization. The packages were incubated after exposure. They were then opened and examined for colony growth.

RESULTS. The number of recontaminated packages per test group (n = 50) depended on the microbial bioload (defined as the number of colony-forming units per plate) to which the packages were exposed and on the size and number of decreases in atmospheric pressure. Results of multiple regression analysis showed a significant increase in the number of recontaminated packages in correlation with the product of the values for microbial bioload and the size and number of decreases in atmospheric pressure. When we analyzed the probability of recontamination of wrapped medical devices after 2 reductions in atmospheric pressure (30 millibars each) and with a surface microbial load of 10 colony-forming units per 64 cm², we estimated that the frequency of recontamination was 1 : 100,000.

CONCLUSION. Multiple regression analysis showed that the proposed microbial challenge test is suitable to determine the probability of package recontamination at the 1 : 1,000,000 level.

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serting petri plates filled with nutrient agar into the packaging unit prior to sterilization. After sterilization and exposure to experimental test conditions, the packaging is only opened after incubation, to determine the number of colonies formed. To obviate the statistical difficulties, we developed an exposure chamber method. This microbial challenge test method is designed to test porous packages under conditions that enhance the probability that the passage of microorganisms through the packaging material will be detected. The test is a whole-package test that involves placing the packaging inside the chamber and exposing it to a defined aerosol of microorganisms and to a number of decreases in atmospheric pressure that simulate meteorological highs and lows or temperature-dependent pressure changes. On the basis of these data, the probabilities of contamination were calculated in relation to environmental factors, including the microbial bioload that may be present in the storage area and changes of atmospheric pressure that could arise as a result of the effects of weather (ie, changes of 1050 to 950 hPa in 12 hours) or transport in an elevator.

METHODS

Heat-sealable transparent pouches from 3 manufacturers (Steriking, Wipak Walsrode; Stericlin, VP; and Brömeda, Amcor Flexibles Deutschland) that are recommended for packing individual instruments and smaller sets were used as packaging material. These products consist of sterilization paper (70 g/m²) and transparent plastic film. Uncovered thermoresistant petri dishes (diameter, 90 mm) filled with 25 mL of Sabouraud agar (Oxoid) were inserted into the packages. After sealing, the packages were sterilized at 121°C for 15 minutes with a 30-minute drying cycle (ELV autoclave; Systec). Sterilizer runs were monitored periodically (ie, every 2 weeks) with biological steam indicators (Spore-O-Check; ATI). After the agar had cooled to below 40°C and solidified, the test packages were removed from the autoclave.

The stainless steel exposure chamber (breadth, 80 cm; length, 50 cm; height, 60 cm) contained a port for attachment of a nebulizer (Pari Juniorboy N; PARI) and a port for a vacuum pump (Sartorius MD2; Sartorius). The bottom section contained trays to accommodate sterilized packaging materials. By use of different test sets, the atmospheric pressure was periodically reduced by 0, 25, 50, and 75 millibars (mbar), respectively. One cycle lasted 4 or 5 minutes. Humidity and pressure changes over time were registered.

By use of the nebulizer, baker’s yeast (Saccharomyces cerevisiae; concentration, 10⁷–10⁸ organisms/mL) was used to periodically produce a microbial aerosol (5 mL per hour) in the exposure chamber; when the valve was opened to equalize the pressure difference, the aerosol passed through the valve and was dispersed into the chamber. As specified by the manufacturer, the median diameter of particles was 3.9 μm (64% of particles were <5 μm in diameter). To minimize possible risk to the health of the technical staff, S. cerevisiae was used as the test organism. As controls, 6 uncovered petri dishes filled with nutrient agar were exposed on the trays in the chamber, to detect surface microbial loads during the test. After exposure, the test packages and the settle plates were incubated at 32°C for 48 hours. After incubation, the packages were opened, and the results were recorded as the number of colony-forming units per plate. As specified by the European standard EN 868-1, if colony growth could be detected, then a dye penetrant solution was applied locally to the sealed edge of the packaging to identify leaks. After the plastic film was cut, several drops of a 0.15% solution of rhodamine B in 5% propanol were applied to the inner side of the sealed edge. If, after 15 minutes, penetration of the dye solution could be observed, then the seal integrity was considered to be damaged. The surface microbial load at the
end of exposure was calculated as the mean number of colony-forming units on the settle plates (area measured in square centimeters).

Multiple regression analysis was used to calculate the correlation between the number of recontaminated packages and exposure factors, such as surface microbial load, strength of atmospheric pressure changes, and number of these pressure changes. We used WinSTAT (version 2003.1; R. Fitch Software) for statistical analysis.

RESULTS

The increase in relative humidity and the cycles of atmospheric pressure change over the course of 70 minutes in the chamber under exposure conditions are shown in Figure 1. Owing to the periodical exposure to the microbial aerosol, the humidity increased from 60% to 80% during the test. Under these conditions of relative humidity, no wet packages were seen in the various tests.

There are 2 ways of introducing the petri dishes into the packages: one way is to place the agar surface under the transparent plastic film, and the other is to place the agar surface beneath the paper side. We used both of these methods in a set of tests using 48 pressure changes (75 mbar) and a mean surface microbial load of approximately 1500 cfu per 64 cm². When the petri dishes were placed in the transparent pouches (Stericlin) with their uncovered surface under the paper side, 82 of 237 packages were found to be nonsterile. However, only 3 of 166 packages were recontaminated when the uncovered dishes were positioned under the transparent plastic film. No leaks of the seal edges could be seen in any nonsterile pouch. Plates inside the pouches had 1-4 colonies per plate. These results indicate that it is the paper side and not the plastic side that enables the passage of microorganisms. In all subsequent tests, the uncovered surface of the petri dishes was, therefore, placed directly under the paper side of the packaging, to detect nonsterility more sensitively.

To compare the barrier function of transparent pouches from 2 different manufacturers (Steriking, Wipak Walsrode; and Stericlin, VP), 4 equivalent sets of 50 packages at a time were tested. The test conditions were as follows: the exposure time was 4 hours, there were 12 pressure changes per hour, and there was a 75-mbar difference in atmospheric pressure (Figure 1). Almost identical results were obtained for both sets of packages: of 100 packages tested, 11 of the Stericlin packages and 12 of the Steriking packages were nonsterile. There were 1-4 colonies on the plates inside the pouches. The mean surface microbial load on the settle plates exposed in the chamber was 1784 cfu per 64 cm².

In the same way, another pair of transparent pouches from different manufacturers (Stericlin, VP; and Brômeda, Amcor Flexibles Deutschland) was compared. We found that 4% of the Brômeda transparent pouches were nonsterile (Figure 2). However, 16% of Stericlin pouches were nonsterile. The mean surface microbial load was 2161 cfu per 64 cm².

To compare the transparent pouches (Stericlin) used in a single-layer package with those used in a double wrapping, 4 runs each with 25 single-layer package and 25 double-layer packages were performed. The test conditions were as follows: the exposure time was 4 hours, there were 12 atmospheric pressure changes per hour, and there was a 75-mbar difference in atmospheric pressure. The mean surface microbial load was 1067 cfu per 64 cm²; 4% of the 100 single-layer packages and none of the 100 double-layer packages were nonsterile.

In a further series of tests, we examined the effect of the number of pressure changes at constant pressure difference of 75 mbar. The number of periodic pressure changes was 0, 6, 12, 24, 36, and 48. Packages of 100 pouches (Stericlin) were used for each test group. Figure 3 shows that the number of recontaminated packages increased as the number of atmospheric pressure changes increased from 0 to 48. The microbial loads were 533-1784 cfu per 64 cm².

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To evaluate different pressure changes and different microbial loads as variables, we exposed 50 packages each to 48 discrete pressure changes (0, 25, 35, 50, and 75 mbar) and to microbial aerosols of high (10⁸ organisms per mL) and low (10⁷ organisms per mL) concentration. When packages were exposed to increasing mean microbial loads (370 vs 1330 colony-forming units per plate) and incremental atmospheric pressure reductions of 0, 25, 35, 50, and 75 mbar, the number of recontaminated packages increased correspondingly (Figure 4).

On the basis of these dose-response data, a multiple regression analysis (stepwise procedure) was performed with colony-forming units, pressure changes, the number of pressure changes, and the product of these 3 predictors as independent variables and the number of recontaminated packages as the dependent variable. The results of analysis showed a highly significant (P < .001) regression coefficient for the product of colony-forming units, pressure changes, and the number of pressure changes.

As shown in Figure 5, the risk of nonsterility can be assessed for different conditions and parameters, such as exposure to precipitated airborne microorganisms (colony-forming units per surface area), weather-dependent atmospheric pressure changes, and the number of pressure changes. If the total surface contamination load and the number and extent of weather-dependent pressure changes is known, the probability that there will be one contaminated item can be specified. When we analyzed the probability of recontamination of wrapped medical devices after 2 reductions in atmospheric pressure (30 mbar each) and with a surface microbial load of 10 cfu per 64 cm², we estimated that the frequency of recontamination was 1 : 100,000.

**DISCUSSION**

The recommended times and temperatures for steam sterilization are based on thermal-death curves obtained by plotting the log of surviving spores of thermophilic test organisms, such as *Bacillus stearothermophilus*, against the exposure time. Under the assumption that sterilization starts with 10⁵–10⁶ organisms and that the overkill procedure is used, the theoretical probability that there is a viable microorganism present on or in the device is less than or equal to 1 × 10⁻⁷. Exposure to a temperature of 121°C for 15 minutes is commonly used for steam sterilization. In contrast to sterilization, there is scant scientific information on the properties of packaging as barriers to different influences, such as movements, shocks, repeated handling, load of airborne microorganisms, and atmospheric pressure changes. Several published studies investigating various packaging materials have focused on storage time (ie, shelf life) only.²³,⁵,⁸,⁹ Butt et al. examined the contamination rate for 7200 packages and showed that the rate did not differ significantly between controls and packages that were stored for up to 52 weeks. An event-related expiration date approach is suggested by Webster et al. On the basis of the results of their study with 152 packages and 262 test samples, they concluded that products correctly wrapped and sterilized remain sterile unless the wrapping is damaged. On the other hand, using a standardized protocol of frequently observed events in the sterilization area, Widmer et al.¹⁰ noted that 8.3% surgical trays that had undergone routine treatment prior to use were contaminated, compared with 2.5% control packages. In our experience, the packaging used for steam sterilization is not impervious to microorganisms, by definition. Therefore, we have to consider all factors and activities (eg, shocks, airborne microorganisms, and atmospheric pressure gradient movements) that could
influence the sterile integrity of the packaging as “events.” Time-related expiration dating for a defined type of packaging should be objectively established at an acceptable level on the basis of experimental data. The European standard EN 556 defined this level as 1 : 1,000,000 for the likelihood of non-sterility. Our data demonstrate that an enclosed device remains sterile at this 1 : 1,000,000 level only when defined transparent pouch packaging is used and when the environmental conditions are checked and maintained within defined limits for airborne load of microorganisms and pressure changes. As our findings demonstrate, transparent pouches used as double-layer packages provide a sufficient assurance of sterility in routine clinical settings. We used a relatively large organism that was approximately 10 μm in diameter for exposure, so our results may underestimate the risk of nonsterility, compared with that in the real environment, where bacteria and their endospores, which are approximately 2-5 μm in diameter, are frequently present.

Tests performed under practical conditions with a limited sample size may be helpful as a random search, but they cannot substitute for laboratory investigations. The validation of the sterile integrity of wrapped medical devices can be rendered more precise only by using dose-response data. In the routine clinical setting, data concerning the total surface contamination load and other influences, such as weather-dependent pressure changes, cannot be obtained. To establish appropriate safety measures that prevent damage to health, the manufacturer should provide whole-package integrity data obtained from microbial barrier testing on the basis of dose-effect relationship, as demonstrated in this study. By use of these data and under the assumption of a defined exposure scenario, the user could make a risk assessment based on strong scientific evidence for one contaminated item.

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