

Measurement of the microbial barrier effectiveness of sterilization containers in terms of the log reduction value for prevention of nosocomial infections

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Background: The microbial barrier properties of 216 sterilization containers of 4 central sterile supply departments of different hospitals were measured using a microbial challenge test.

Methods: Uncovered thermoresistant plates filled with Sabouraud agar were placed on the base of the containers prior to sterilization. After sterilization, the containers were exposed to a defined microbial aerosol and periodic atmospheric pressure reductions of 10 to 70 hPa in an exposure chamber. After exposure and incubation of the entire containers, colony growth was registered as colony forming units (CFU) on the plates and the control plates to calculate the barrier effectiveness in terms of the logarithmic reduction value (LRV).

Results: Two out of 11 standard containers with paper filters and 9 out of 79 containers with textile filters showed no growth on the plates. The mean colony numbers were 222 CFU/600 cm² (standard container with paper filter) and 209 CFU/600 cm² (container with textile filter). Fourteen out of 15 containers with permanent plastic filters did not show any growth on the plates. No recontamination was observed in 18 of 111 half-size containers. The mean colony numbers of the recontaminated half-size containers were 110 (paper filter) and 34 CFU per 300 cm² (textile filter). The LRVs of the full-size and half-size containers tested ranged between 1.08 and >4.

Conclusion: As shown in this study, the microbial barrier effectiveness of sterilization containers in the routine clinical setting can be tested with a quantitative microbial challenge test at intervals of 1 year to eliminate defect or ineffective containers as potential causal factors for nosocomial infections. An LRV of >4 should be envisaged as the target assurance level. (Am J Infect Control 2006;34:285-9.)

Sterilization containers are widely used as medical sterilization packaging systems. Containers are made of stainless steel, aluminum, or high-temperature plastics. A filter or valve system is incorporated into the lid and/or the base of the container to enable air evacuation and adequate sterilant introduction and to act as a barrier to microorganisms during transportation and storage. The lids contain gaskets that encircle the sealing portion of the lid. Sterilization containers are reusable devices, but various factors endanger the package integrity during aging: the latching mechanism that secures the lid to the base can deteriorate, and the lid seal can be impaired by repeated use of heat and by mechanical stress. Rivets, bolts, and nuts

can be loosened. The transportation, the use, and the reprocessing of the containers require much handling and can endanger their integrity. Therefore, each container should be inspected before preparing the containers for use to ensure that the sealing surfaces are not dented or chipped. The filter mechanism and the filter material such as paper, long-term textile filters, or permanent filters should be checked for integrity and for absence of holes.¹ However, this visual examination is inaccurate and not really adequate to establish the effectiveness of the microbial barrier properties of the filter used, of the filter mounting, of the locking mechanisms, and of the entire container as a unit.

In addition to visual examination, the microbial barrier properties of sterilization container systems should be periodically evaluated with an appropriate test method that simulates exposure under the most rigorous conditions such as highly contaminated environment and atmospheric pressure changes. The exchange of air between the inside of the container and the environment entails a risk of recontamination. This is because a lower pressure inside the container will be compensated by a flow of air from the environment into the container. Pressure differences are produced by transfer to an area with a positive pressure

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(eg, an operation room with hyperbaric pressure), by change of weather, and by transport in an elevator.

The European standard EN 556 defines sterility for a terminally sterilized medical device as the theoretical probability that a viable microorganism will be present on/in the device is equal to or less than 1×10^{-6} .² To measure the capacity of a packaging system to prevent microorganisms from entering, an exposure chamber method was developed in our laboratory as a microbial challenge test.³ This is a whole package test that consists of placing the packaging inside the chamber and of exposing it to a defined aerosol of microorganisms and to a number of periodic atmospheric pressure reductions that simulate meteorologic highs and lows or temperature- and transportation-dependent pressure changes.

The purpose of this study was to examine the barrier properties of sterilization containers used in 4 central sterile supply departments for several years. Furthermore, the barrier functions of brand new containers were compared with those of double-layer paper wrapping and of nonwoven material. In this study, the microbial challenge test method developed to ascertain penetration of airborne bacteria through the medical device packaging system after sterilization was applied to the sterilization container for the first time.

METHODS

Materials

To check as large as possible an area of the container, heat-stable plates (15 × 20 cm) filled with Sabouraud agar (art. no CM0041; Oxoid GmbH, Wesel, Germany) were placed into the containers prior to sterilization. After sterilization and exposure to experimental test conditions, the containers were opened only after completion of the incubation to determine the number of colonies formed.⁴

The exposure chamber (breadth 80 cm, length 50 cm, height 60 cm) was constructed of stainless steel. The chamber contained a port for attachment of the nebulizer (Pari Juniorboy; PARI GmbH, Starnberg, Germany) and a port for the vacuum pump (Sartorius MD2; Sartorius, Goettingen, Germany). The bottom section contained trays to accommodate sterilized packaging materials. The atmospheric pressure was reduced/increased by 10 to 70 hPa every 5 minutes using 2 time switches, which control the nebulizer, and the control valve for the inflow of the microbial aerosol and the turning on and off of the vacuum pump. Using the digital pressure measuring instrument testo 525 (Testo GmbH & Co., Lenzkirch, Germany), the differential pressures were continuously monitored. To prevent the risk of condensation on the packaging, the relative humidity was registered using a Hygrolog

with Hygroclip (Rotronic, Switzerland). To prepare a microbial suspension of *Saccharomyces cerevisiae*, 1 g baker's yeast (Dr. Oetker AG, Switzerland) was dissolved in 100 mL sterile water. The microbial concentration was determined by the dilution test. Using the nebulizer, 1, 5 mL aliquot at a concentration of 10^8 organisms/mL was applied to produce an aerosol in the exposure chamber for 1 hour. This application of the microbial suspension was repeated for the exposure of 2 hours. When the valve was opened to eliminate the pressure difference, the aerosol passed the valve and dissipated in the chamber. To rule out a possible risk for the health of the technical personnel, *S cerevisiae* was used as test organism. Six uncovered petri dishes (diameter 90 mm) with Sabouraud agar were exposed as controls on the trays in the chamber in different places to detect the mean surface microbial load during the test. After exposure, the containers and the control plates were incubated at 32°C for 48 hours. After each test run, the chamber was cleaned with a solution of 80% ethanol in water. Results were recorded as colony-forming units (CFU) per cm² after incubation. If the microbial challenge in any run varied more than ± 0.5 log from the average, that run would be repeated.

Calculation of the log reduction value

The ability of the containers to resist the passage of microorganisms was expressed as the log-reduction value (LRV) calculated by the following equation⁵:

$$\text{LRV} = \log_{10} N_0 - \log_{10} N_1$$

where:

N_0 = average surface microbial load determined from the control plates (CFU/cm²)

N_1 = number of microorganisms passing through packaging 1 (CFU/cm²)

If $N_1 < 1$, then LRV was expressed as $> \log_{10} N_0$

In total, 105 standard containers (58 × 28 × 15 cm) and 111 half-size containers (29 × 28 × 25 cm) from 4 central sterile supply departments of different hospitals were tested. The test conditions were as follows: exposure time 2 hours, 12 atmospheric pressure changes per hour, 70 hPa pressure difference, microbial aerosol of 2 × 5 mL suspension of baker's yeast. The containers were furnished with paper filters, textile filters, or permanent plastic filters.

To establish the effects of different pressure reductions as independent variable, we exposed each of 12 containers to 24 periodic pressure changes of 10, 25, and 70 hPa, respectively, and to an aerosol of 10^8 organisms per mL microbial suspension. Five brand new sterilization containers with permanent filter were investigated to compare the container system with

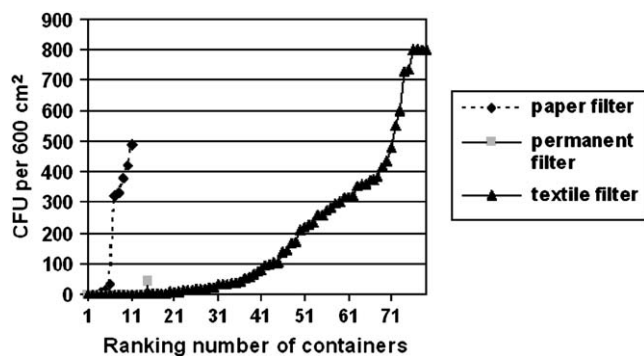


Fig 1. Cumulative distribution of 105 sterilized standard containers with increasing CFU per 600 cm² after exposure to the microbial challenge test.

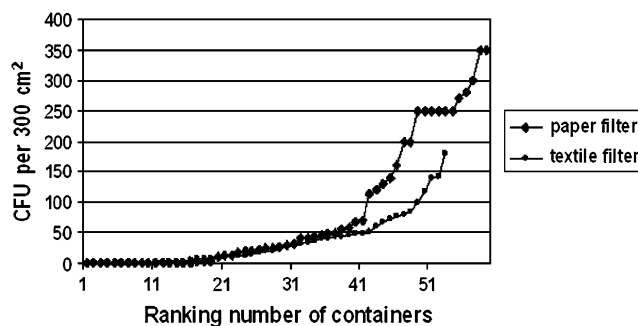


Fig 2. Cumulative distribution of 111 sterilized half-size containers with increasing CFU per 300 cm² after exposure to the microbial challenge test.

flexible wrapping materials. Wrapping paper crepe and nonwoven wrapping measuring 90 cm × 90 cm served as double-layer packaging materials according to EN 868-1 (art. no. 4FZBH113003 and 4FZBH216006; VP GmbH, Feuchtwangen, Germany).⁶ Wire baskets measuring 220 mm × 550 mm were used for packaging. The packaging was of a diagonal design in accordance with DIN 58953, supplement: part 10 (DIN Deutsches Institut für Normung e.V., Berlin, Germany), and fixed with adhesive tape. The test run was performed twice. Sterilization was carried out with an ELV autoclave (Systec GmbH, Wetztenberg, Germany) with the following program: 121°C, 2 bar, sterilization time 15 minutes, slow cooling.

RESULTS

The 105 standard containers could be categorized as 11 containers with paper filters, 79 containers with reusable textile filters, and 15 containers with permanent plastic filters. Two, 300-cm² plates filled with Sabouraud agar could be placed in each of these containers. Figure 1 shows the cumulative distribution of containers with increase of CFU per 600 cm². Two out of 11 containers with paper filters and 9 out of 79 containers with textile filters remained without colony growth on the plates. The mean LRVs were >4.1 (sterile container with paper filter) and >3.9 (sterile container with textile filter).

The mean number of colonies on plates in containers showing recontamination was 222 CFU/600 cm² (range, 1-490; containers with paper filters) and 209 CFU/600 cm² (range, 1-800; containers with textile filter). Fourteen of the 15 test containers with permanent plastic filters did not show any growth on the plates. Their mean LRV was >4.04. In 1 out of the 15 containers, 1 filter had been damaged by a small incision of approximately 1 cm, which was hidden by the

filter retainer. This small defect resulted in 48 CFU on the plates.

Furthermore, the barrier effectiveness of 5 brand new standard containers with permanent plastic filters was compared with that of double-layer paper wrapping and nonwoven material. No nonsterile containers or nonsterile double-layer packages could be observed in any case. The mean LRV was >4.25.

One hundred eleven half-size containers were examined. They comprised 58 containers with paper filters and 53 containers with textile filters. The results obtained with these containers are shown in Fig 2.

No recontamination was observed in 10 containers with paper filters (mean LRV >4.0) and in 8 containers with textile filters (mean LRV >3.9). The containers with recontamination showed a mean colony number of 110 CFU/300 cm² (range, 1-350; paper filter) and 34 CFU per 300 cm² (range, 1-180; textile filter). The cumulative distributions of LRVs of the full-size and half-size containers with paper and textile filters are shown in Fig 3.

The standard and half-size containers with recontamination that were examined showed visible defects such as fissured or loosened lid seals in several cases. No clear defects could be correlated with the microbial findings in other instances.

Pressure changes in the clinical setting are more likely to be slight. Under these conditions, the probability of recontamination of the containers could be very low. It was therefore of interest to obtain experimental results after exposure to lower pressure differences. The first 12 containers from the same hospital were examined using 24 pressure reductions of 70, 25, and 10 hPa. The investigation with each pressure difference was performed twice. Only 4 of the containers showed an LRV between 3.4 and >4, which demonstrates a high barrier efficacy (Fig 4), whereas the other containers tended to show a higher colony number after exposure and lower LRVs between 1.8 and 2.5.

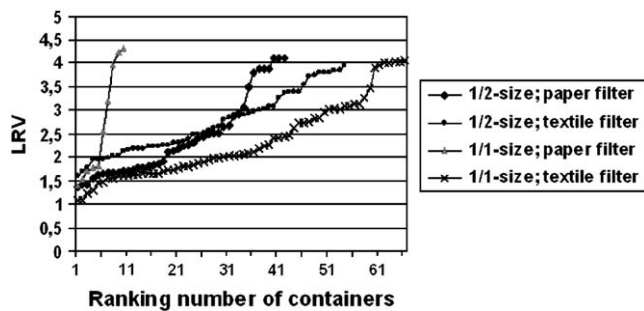


Fig 3. Microbial barrier effectiveness of the standard and half-size containers in terms of log-reduction values (LRV), cumulative distribution.

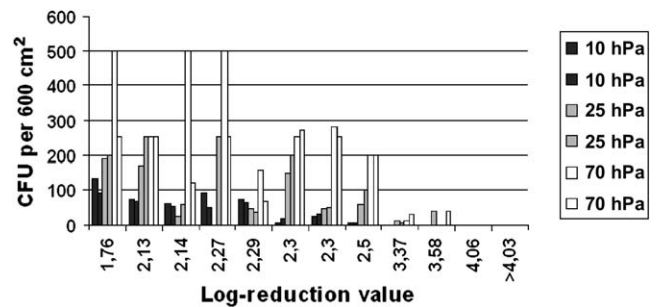


Fig 4. Ranking of 12 containers for LRVs after exposure to the microbial challenge test using 2 runs of 24 pressure differences of 10, 25, and 70 hPa.

DISCUSSION

Consistent with the general trend toward global regulatory harmonization for medical devices, the efficacy of procedures such as decontamination (sterilization, disinfection) or packaging has to be documented with quantitative test results. Recontamination can take place in the period of time between sterilization and use of the sterilized objects. However, a suitable and universally accepted method of demonstrating the effectiveness of the microbial barrier properties of terminally sterilized medical devices has not been introduced.

The existing standards for the packaging material generally relate to partial aspects of the packaging unit such as material properties, tightness of sealing closure, and others. A method for testing the individual components of a container package consists in generating a negative pressure in the container via the filter passages provided in the cover of the container (EN 868-1, Appendix G).⁶ The sealing tightness between the lid and the container base can be tested by registering a renewed increase of the pressure. However, this method only investigates the seal between the container base and the lid but not the microbial barrier characteristics of filters, the filter retainer, and the container as a whole.

In the method described by Junghannss et al, a test chamber is used into which the containers can be inserted after sterilization and filled with petri dishes containing nutrient agar.⁷ A microbial aerosol was introduced into the test chamber using a spray bottle. Via pipe connections, air was withdrawn with a pump hose. Afterward, the containers were opened, and the agar plates were removed and then incubated for colony growth. Junghannss et al⁷ examined the microbial barrier properties of 20 containers that had been in daily use for several years in 4 hospitals and were subjected to approximately 1400 to 2300 steam

sterilization cycles during this time. The results of this study showed a microbial retention capacity of at least 99.99%, corresponding to an LRV of 4. An important divergence of the test conditions of Junghannss et al from those of this study could be the reason for the difference in the results: Junghannss et al used a spray can to produce a microbial aerosol and exposed the containers to the microbial aerosol before the withdrawal of 18 L air, whereas, in our study, the movement of air induced by the pressure gradients and the exposure to the microbial aerosol generated by the nebulizer (particle size: 64% <5 μm in diameter) took place at the same time. The method of Junghannss et al is not suitable for testing containers in routine hospital practice because it requires a suction connection to the container.

Several publications with different packaging materials have focused on shelf-life only using a method that entails opening the packaging after sterilization and storage; a sample such as glass rods, stainless steel coupons, copper rods, screws, or dental instruments is then transferred to a culture tube.⁸⁻¹² However, this method has some drawbacks such as a risk of accidental contamination, no matter how careful the technique, or the use of relatively small samples in relation to package surface. Finally, only the relation of nonsterile to sterile samples could be established, not the log-reduction value.

Test data of kinetic studies such as the survivor curve and calculation of D-value must be furnished according to the European standards and meet requirements for terminally sterilized medical device packaging systems. On the basis of these data, the sterilization conditions for a sterility assurance level of 10^{-6} must be fulfilled. For a carrier inoculated with 10^6 spores, a 12-log reduction in the populations must be attained. The importance of routinely monitoring the quality of sterilization procedures has been established. Even though there are no reports

indicating that failures of the packaging integrity led to nosocomial infections, a strong theoretic rationale supports the need for microbial monitoring of packaging systems.

In this way, evidence for the sterility of medical devices in their final packaging should also be provided at intervals of 1 year to show that the barrier effectiveness of the packaging does not fall below a defined assurance level. It is noteworthy that the 14 of 15 standard containers with permanent plastic filter did not show any growth on the plates. These practically new containers as well as the 5 brand new standard containers with plastic filters resulted in high LRVs of >4. From our point of view, there are 2 possible reasons why these containers mostly showed no colony growth. First, they had been in use for a maximum of 2 years. Therefore, their integrity was not impaired by mechanical stress or ageing (by comparison, the year of acquisition of the standard containers with textile filters was between 1980 and 1990). Second, the permanent plastic filter is probably a more effective microbial barrier than the paper or textile filter.

Figure 4 shows that the impact of pressure changes influences the number of CFU, as would be expected. Therefore, we recommend that a defined pressure between 25 and 70 hPa for routine examination be used. According to the results of the present study, an LRV of 3.5 suffices to confirm the sterile integrity of the containers. However, an LRV of >4 should be envisaged for sterilization containers to prevent nosocomial infections. This value indicated no colony growth after exposure in the quantitative microbial challenge test and demonstrated relatively high barrier properties. The regular examination of containers entails technical expertise and is expensive. However, only the use of suitable and validated procedures for routine checks can ensure that processed medical devices do not pose any health risks to patients.

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