original article

# Determination of the Efficacy of Sterile Barrier Systems Against Microbial Challenges During Transport and Storage

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objective. The sterility assurance level of $10^{-6}$ is an established standard that defines the quality of sterile products. The aim of the present study was to develop a method that correlated the results from microbial-barrier testing of flexible sterile barrier systems with the estimated microbial challenge that the package encounters during storage and transport.

methods. The effectiveness of microbial-barrier packaging was determined by the use of an exposure chamber test with 20 periodic atmospheric pressure changes of 50 and 70 hPa. Flexible peel pouches were used as sterile barrier systems. The logarithmic reduction value of a sterile barrier system was calculated on the basis of the experimental results and compared with the logarithmic reduction value required for the microbial challenges to maintain sterility during transport and storage.

results. For pouches made of paper and plastic-film material, a logarithmic reduction value of 5.4 was obtained on the basis of 30 of 99 plates becoming nonsterile after being exposed to a 50 hPa difference in periodic atmospheric pressure changes. For pouches made of paper and plastic-film material, a logarithmic reduction value of 5.2 was obtained on the basis of 48 of 100 plates becoming nonsterile after being exposed to a 70 hPa difference in atmospheric pressure. For pouches made of nonwoven and plastic-film material, logarithmic reduction values of 6.38 (ie, 3 of 99 plates became nonsterile after being exposed to a 50 hPa pressure difference) and 6.07 (ie, 3 of the 99 plates became nonsterile after being exposed to a 70 hPa pressure difference) were obtained. Calculating an expected microbial challenge during transport and storage that requires barrier properties corresponding to a logarithmic reduction value of 5.83 and taking the sterility assurance level into account, we found that only the nonwoven pouches fulfilled the European standard EN 556-1.

conclusions. Using the data obtained in a microbial exposure test with a specified flow rate of a bacterial aerosol, we found that the effectiveness of the sterile barrier system against the actual microbial challenge can be examined and evaluated at the sterility assurance level of $10^{-6}$.

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An essential requirement for medical sterilization packaging systems is that they must maintain the sterility of the enclosed objects for the specified shelf life during transport and storage. In practice, there are very different kinds of sterile barrier systems (eg, flexible peel pouches of paper or nonwoven material and plastic film, porous medical-grade paper sterilization bags, and rigid reusable aluminum or stainless steel sterilization containers). The specific form of the medical product, its intended use, the sterilization procedure envisaged, and the transport conditions all influence the choice of materials for packaging systems.[1] In hospitals and healthcare facilities, sterilization by means of steam, ethylene oxide, or oxidizing processes is widespread. The sterile barrier system has a permeable component that permits the permeation of the sterilizing agent. For example, the paper for pouches must comply with the requirements of the European standard EN 868-3 (ie, the average pore diameter must be less than or equal to 35 $\mu$m, with a maximum pore diameter of 50 $\mu$m).[2] However, because microorganisms may be smaller than these size limits, there is a risk that bacteria will penetrate the packaging. The microbial challenge to packaging varies greatly on the basis of the environmental conditions. It is affected by many factors, such as storage conditions (cleanliness and temperature) and transport, which may be associated with a high microbial load and/or meteorological pressure and temperature changes.

By means of the sterility test, several studies have attempted to show that the packages adequately protect the product from recontamination during storage and that they maintain sterility.[3-6] However, the sterility test suffers from different methodological limitations. It is true that "sterile" is an absolute term, but the confirmation that any product is sterile can only be defined in terms of a probability function. Accordingly, sterility is defined by the minimum acceptable sterility assurance level, which limits the probability of a nonsterile unit to $10^{-6}$.[7,8] A total of 3.8 million items had to be tested to confirm that a product batch was sterile in conformity with the sterility assurance level (with 95% confidence in-

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tervals [CIs]). Furthermore, sterility tests entail the risk of adventitious microbial contamination that results in false-positive readings. With reference to these limitations, the US Food and Drug Administration stated that "sterility tests are not recommended as a component of a stability program for confirming the continued sterility throughout a product's shelf life or dating period. Alternative methods may be more reliable in confirming the integrity of the container and closure system as a component of the stability protocol for sterile products."[9(p2)]

In previous papers, we described a quantitative method for measuring the barrier efficacy of the entire final packaging, by means of the exposure chamber test.[10,11] The aim of the present study was to develop a method that correlated the results from microbial-barrier testing of flexible sterile barrier systems with the estimated microbial challenge that the package encounters during storage and transport. This procedure was intended to establish whether the barrier properties of the packaging system were sufficient to maintain sterility under the specific conditions of transport and storage at a sterility assurance level of $10^{-6}$.

## METHODS

Two types of flexible peel pouches were used as medical packaging systems. One type consisted of a plastic film on one side and paper on the other. The other type consisted of a plastic film on one side and nonwoven material made of high-density polyethylene fibers on the other. As declared by the manufacturers, the packaging materials used were capable of withstanding steam sterilization at 121°C. Uncovered thermoresistant plates (diameter, 90 mm) filled with 25 mL of casein-peptone soymeal peptone agar (Oxoid) were wrapped with these peel pouches. One test series comprised packages in which the uncovered agar plates were placed with the open side under the paper or the nonwoven surface. In another test series, the open sides were placed beneath the plastic-film side. After sealing, the packs were sterilized at 121°C for 20 minutes with a 30-minute drying cycle (ELV autoclave; Systec). Sterilizer runs were monitored periodically 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.

An exposure chamber with a capacity of 0.24 $m^3$ was equipped with a nebulizer (Pari Juniorboy; PARI) and vacuum pump (Sartorius MD2; Sartorius). The pressure in the chamber was measured digitally with a pressure meter (Testo 525; Testo). The atmospheric pressure was periodically reduced by 50 or 70 hPa. Each cycle period lasted 6 minutes. Humidity and pressure were continuously registered. By means of the nebulizer, 5 mL of a suspension of *Micrococcus luteus* at a concentration of $10^8$ organisms/mL was applied to produce a microbial aerosol in the exposure chamber. When the valve was opened in order to equalize the difference in atmospheric pressure (hereafter, "pressure difference"), the aerosol passed the valve and dispersed into the chamber. Six uncovered settle plates with nutrient agar were exposed as controls on the trays in the chamber, to detect surface microbial loads during the test.

For each test group, 100 packages were used. The packages were subjected to 20 periodic atmospheric pressure changes in 2 hours. For 1 test run, 7–8 packages from 4 test groups per pressure difference (at most, 32 packages) could be placed in the exposure chamber; 13 runs each were needed for the tests with 50 and 70 hPa pressure differences.

After exposure, the packages were incubated at 36°C for 48 hours. Microbial growth was reported as colony-forming units (cfu) per surface area; 95% CIs were calculated for the number of nonsterile packages per test group and for the total microbial count.

If colony growth was observed, a dye penetrant solution was applied to the sealer edge of the packaging to identify any leaks. If there was a leak in the seal edge, the reading was left out of consideration.

The effectiveness of the microbial-barrier packages was expressed as the log reduction value (LRV). The LRV was calculated using the following equation:

$$LRV = \log N_0 - \log N_1,$$

where $N_0$ is the calculated mean number of bacteria present in the total volume of air passing through the porous packaging system during the 20 periodic atmospheric pressure changes of 50 or 70 hPa, respectively. The mean airborne bacterial count per $cm^3$ in the exposure chamber was calculated by means of the consumption of the bacterial suspension and the output of the aerosol during the pressure changes. In our study, an air sampler was not employed to monitor the bacterial concentration in the exposure chamber. However, an all-glass impinger was used after these experiments, to sample and monitor the airborne microorganisms in the exposure chamber. $N_1$ is the number of bacteria reported as cfu on the plates in the packaging after incubation. The volume of air in a package was approximately 120 $cm^3$. The volume of air passing through the porous material of the packaging as a result of a pressure change of 50 hPa could be calculated as 5.64 $cm^3$. A total volume of 112.8 $cm^2$ was obtained for 20 periodic pressure changes. For a 70 hPa atmospheric pressure change, the volume of air passing through the packaging was 7.76 $cm^3$.

Using 2 examples, we calculated the requisite barrier properties of a package in terms of its LRV by estimating both the volume of air potentially penetrating into the packaging during transport and storage and the airborne microbial load ($N_{estimated}$) of this volume. A safety factor (SF) of $10^6$ was added in order to attain the minimum acceptable sterility assurance level of $10^{-6}$. On the basis of these assumptions, the required effectiveness of the microbial-barrier packaging in terms of $LRV_{required}$ was calculated as follows:

$$LRV_{required} = \log N_{estimated} + \log SF,$$

where $N_{estimated}$ is the estimated number of bacteria present in the total volume of air passing through the porous part of the packaging system during different stages and time periods of storage and transport.

In example 1, the following conditions were specified: storage of the packages in an air-conditioned area of a central sterilization department for 100 days, 10 atmospheric pressure changes of approximately 15 hPa, and a total concentration of airborne microorganisms of 30 cfu/m$^3$. In example 2, the following conditions were specified: storage of the packages in a storage room for 100 days with mean daily temperature changes of 15°C, 10 atmospheric pressure changes of approximately 15 hPa, and a total concentration of airborne microorganisms of 1,000 cfu/m$^3$.

Examples of stages of transport and storage typically associated with a passage of air through the porous material of the packaging are as follows: changes in the air pressure during storage, changes in the altitude during transport in an elevator, transport routes at different altitudes above sea level, and air passage in the packaging caused by environmental temperature changes.

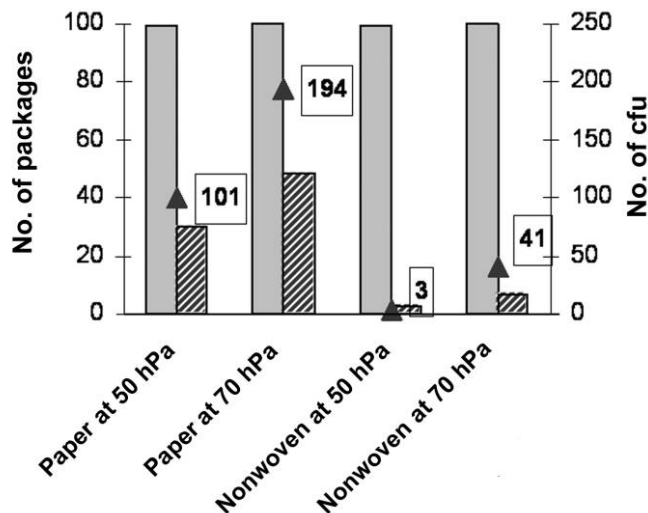


FIGURE. Results obtained to determine the effectiveness of paper and plastic-film pouches and of nonwoven and plastic-film pouches against microbial challenges, by means of the exposure chamber test. *Gray bars,* number of pouches evaluated *(left axis); hatched bars,* number of pouches in which agar plates had microbial growth *(left axis);* arrows and boxed numerical data, total number of colony-forming units (cfu) on the plates.

## RESULTS

After the exposure of the flexible peel pouches (made of the paper or nonwoven component) to pressure changes of 50 or 70 hPa, no microbial growth was seen on the plates if the plastic-film side was placed above the agar surface. This result shows that the plastic-film side was not permeable to microorganisms. Placing the uncovered agar surfaces under the paper side resulted in an increase in the number of recontaminated plates, for both types of pouches and for exposure to both 50 and 70 hPa pressure differences (Figure). For 99 plates covered by paper and plastic-film material (1 pouch was left out of the analysis because of a leak in the seal edge of the packaging), the exposure to a 50 hPa pressure difference resulted in 30 nonsterile plates (95% CI, 20.2–39.0 nonsterile plates). For 100 packages made up of paper and plastic-film material, the exposure to a 70 hPa pressure difference resulted in 48 nonsterile plates (95 % CI, 38.2–57.8 nonsterile plates). For the 99 packages made up of nonwoven and plastic-film material (1 pouch was left out of the analysis because of a leak in the seal edge), recontamination was observed in 3 plates (95% CI, 0–6.3 plates) that were exposed to a 50 hPa pressure difference. For the 100 packages made of nonwoven and plastic-film material that were exposed to a 70 hPa pressure difference, recontamination was observed in 7 plates (95% CI, 2–12 plates). The mean surface microbial load was 3,141 cfu per settle plate (95% CI, 2,999–3,285 cfu per settle plate) for test runs exposed to a 50 hPa pressure difference and 3,516 cfu per settle plate (95% CI, 3,425–3,607 cfu per settle plate) for test runs exposed to a 70 hPa pressure difference.

The volume of air that passed through a package exposed to a pressure difference of 50 hPa was 5.64 cm$^3$. The volume of air that passed through a package exposed to 20 periodic pressure changes was 112.8 cm$^3$. With a calculated mean concentration of microbials in the exposure chamber of $6.5 \times 10^8$ cfu/m$^3$ for tests that employed a 50 hPa pressure difference, the mean airborne load ($N_0$) in a volume of 112.8 cm$^3$ was 73,320 cfu. For tests employing a 70 hPa pressure difference, the calculated mean concentration of microbials was $5.3 \times 10^8$ cfu/m$^3$. Using the mean number of 1.02 cfu ($N_1$) per paper and plastic-film packaging in a test that employed a 50 hPa pressure difference, an LRV of 4.9 was obtained. If the LRV was not calculated by the number of microorganisms entering the packaging but by the number of nonsterile packages (ie, 30 of 99 plates), an LRV of 5.4 was measured. With a 70 hPa pressure difference, LRVs of 4.6 and 5.2, respectively, resulted under the same test conditions with the same type of packages. For pouches made of nonwoven and plastic-film material, LRVs of 6.38 (ie, 3 of 99 plates became nonsterile after being exposed to a 50 hPa pressure difference) and 6.07 (ie, 3 of the 100 plates became nonsterile after being exposed to a 70 hPa pressure difference) were obtained.

The 2 examples with different exposure conditions were used to assess the original microbial challenges during transport and storage. The required effectiveness of microbial-barrier packaging in terms of $LRV_{required}$ that corresponded to example 1 was calculated as follows: an atmospheric pressure change of 15 hPa led to a change in the volume of 1.751 cm$^3$. The total volume of air that passed through the porous pack-

aging was 17.51 $cm^3$, and it was comprised of a microbial load of 0.0005253 cfu or log $N_{estimated} = -3.28$. According to example 1, the requisite barrier properties of the packaging were $LRV_{required} = 2.72$ ($\log 0.0005253 + \log 10^6 = 2.72$). A comparison of the barrier properties in terms of $LRV_{required}$ for the storage conditions found in example 1 (with the results determined experimentally by means of the exposure chamber test) shows that the packages with the paper component as well as those with the nonwoven component unequivocally fulfil the requirement of European standard EN 556-1.

In the case of example 2, the following results were obtained for the volume of air that passed into the packaging. By storing the packages in a storage room for 100 days with mean daily temperature changes of 15°C and by using the formula $V = V_0 (1 + t/273.15)$, where V is the volume after the temperature change, $V_0$ is the volume at the initial temperature, and t is the temperature change in degrees Celsius, we found that $\Delta V_{temperature} = 659$ $cm^3$, where $\Delta V_{temperature}$ is the total volume of air flowing into the packaging during the 100 days due to a change in temperature. There were 10 atmospheric pressure changes of approximately 15 hPa that resulted in $\Delta V_{weather} = 17.51$ $cm^3$, where $\Delta V_{weather}$ is the change in the volume of air due to a change in weather (ie, change in the atmospheric pressure). The total volume of air that was measured flowing into the packaging was 676.5 $cm^3$. The corresponding microbial load at 1,000 cfu/$m^3$ was 0.6765 cfu ($\log 0.6765 = -0.1697$). Calculating the required barrier properties ($LRV_{required}$) according to example 2, we found that $LRV_{required} = 5.83$. Comparing these results with the experimental data, we found that the paper and plastic-film packaging (with LRVs of 5.4 and 5.2 based on 50 and 70 hPa pressure differences, respectively) does not have a sufficient barrier efficacy for the provided transport and storage, according to example 2. However, the nonwoven and plastic-film package with LRVs of 6.38 and 6.07 based on 50 and 70 hPa pressure differences, respectively, clearly fulfils the requirements of European standard EN 556-1.

## DISCUSSION

The microbial load of the ambient air is subject to numerous influences from the environment, such as occupational activities, seasonal and weather-dependent factors, as well as air-conditioning facilities inside buildings. In hospital rooms, the concentration of microorganisms ranges mostly from $10^2$ to $10^3$ cfu/$m^3$, except for highly clean rooms, such as intensive care units or operating theaters.[12] The fraction of the microbial load with a particle size of less than 3.0 $\mu$m varied from 17% to 31% during a 1-year period of investigation. The most frequently isolated species in health-service buildings were gram-positive cocci, such as *Staphylococcus epidermidis* and *Staphylococcus hominis.*[13] The airborne microbial load can be an important factor in the microbial challenge of the sterile barrier systems. A critical remark must be made: the accuracy of the results obtained in the present study is limited, because an air sampler was not used to monitor the bacterial concentration in the exposure chamber.

An expiration date is frequently used by hospitals to assure the sterility of wrapped, sterilized medical products. However, in certain studies,[14,15] it has been shown that "event-related outdating" has been supported, because of its special cost benefits. The conclusion drawn from the results of these studies that address the shelf life of stored sterilized materials was that the packaging remained sterile indefinitely or until an event compromised the integrity of the packaging. Sterility would be maintained if a special event (eg, tears, holes, or moisture) that compromises the integrity of the packaging could not be identified. Therefore, relatively conspicuous changes are regarded as "events." This approach assumes that the packaging is normally an absolutely impermeable barrier for microorganisms and ignores the fact that packaging that is sterilized by means of steam or ethylene oxide has a vapor-permeable and gas-permeable component with mean pore diameters of up to 35 $\mu$m and a required permeability for air at 14.7 hPa of 3 L/minute, for an area of 100 $cm^2$ (eg, EN 868-3).[2] It has to be noted that, in this context, EN standards apply to Europe but not to the United States. As we have shown in previous papers and in the present study, recontamination is, of necessity, not only an event-related process but also always a time-dependent process, in regard to porous medical-grade material.[10,11] It is influenced by the barrier properties of the packaging—for example, the number of layers in the case of packages or the quality of wrapping material—and by ambient conditions, such as the indoor and outdoor microbial content in the air, changes in temperature and atmospheric pressure, and mechanical stress during handling.[16] These studies show that all these environmental factors are effectively "events" and that neither an exclusively event-related date nor an exclusively time-related expiration date is appropriate. The outdating should be performed on the basis of a scientific-based risk assessment that considers the barrier properties of the package, on the one hand, and the appropriate effectiveness of the barrier packaging necessitated by the specific unavoidable environmental influences, on the other. The international standard ISO 11607-1 specifies that the manufacturer of sterile barrier systems shall provide information concerning any known restrictions (eg, environmental conditions) on handling or use, and concerning the frequency and nature of maintenance measures applicable to reusable materials or to preformed sterile barrier systems.[1]

In our study, we showed that the concept of LRV that characterizes the effectiveness of the barrier packaging can be successfully applied to the calculation of the required effectiveness of the barrier packaging that results from the microbial challenges and exposure conditions relevant for transport and storage. In this way, it can be established whether the barrier properties of the packaging are sufficient for the specific microbial challenges, in accordance with the qualitative standard of the sterility assurance level. We therefore recommend that manufactures and users of sterile barrier

systems should present data on the effectiveness of the microbial-barrier packaging. The compatibility of the packaging with the specific physical conditions (ie, temperature and atmospheric pressure changes) and microbiological conditions during transport and storage can then be checked and assessed. Sterile medical products wrapped in porous packaging can be reliably handled in a responsible way only if these data are available.

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