IN QUEST OF STERILE PACKAGING: PART 1

APPROACHES TO PACKAGE TESTING

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Packaging for terminally sterilized medical devices serves several functions. As is the case with most packaging, the design of the package and its component materials must act to protect the contents from hazards that may arise as part of normal distribution and handling. Unlike other products, however, most medical devices are terminally sterilized, and the packages designed for such devices must also fulfill certain functions related specifically to the task of maintaining the sterility of their contents.

Device packages must allow effective sterilization of their contents by whatever sterilization method the manufacturer has specified as appropriate for the device. To ensure effective sterilization processing of devices that require gaseous sterilization methods, for instance, the package design usually incorporates porous materials. Once a packaged device has been sterilized, the primary package must also act to maintain the sterility of that device. Sterility maintenance is of paramount importance to ensuring that a safe and efficacious medical device is provided to the patient at the point of use. However, the approaches and methods used by device manufacturers to test the ability of a package to maintain sterility have been subject to considerable debate.[1–8]

This article reports the results of a preliminary investigation undertaken by the Health Industry Manufacturers Association (HIMA) Sterile Packaging Working Group in order to resolve some of the questions related to the testing of sterility maintenance in medical packaging. The investigation compared the results of a whole-package microbial challenge test versus visual inspection and dye penetration tests on film/film packages formed with intentional seal channel defects.

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LIMITATIONS OF CHALLENGE TESTING

The traditional approach used to assess sterility maintenance has been to perform sterility testing on the package contents after exposure to a variety of environmental stresses over various time intervals. Usually, whole-package microbial challenge testing includes challenging the package with large populations of microorganisms.[9,10] If sterility testing of the package contents does not result in microbial growth, it is assumed the microbial barrier properties of the porous material are acceptable. This approach appears both logical and simple. However, in practice it can be fraught with complications resulting in an incomplete or incorrect assessment.[2,3,6]

Designing a Valid Challenge. One significant limitation of a microbial challenge test for evaluating the barrier properties of an intact package is that it is virtually impossible to maintain control of the microbiological variables while simultaneously incorporating and controlling the range of physical variables that could influence the penetration of a microbe into a package. In addition to the presence of microbes, investigators must also control changes in pressure, temperature, humidity, physical stresses, static charges, and other factors that might affect the outcome. Moreover, test results can also be influenced by such factors as the packaging materials and package shape and size, which the investigator cannot alter.

The chances of establishing a single, properly validated microbial challenge test procedure applicable to a wide range of package designs and packaging materials are very small.

There are several distinct steps that must be performed to validate a whole-package microbial challenge test. First, the nature of the aerosolborne challenge organism must be chosen and a rationale for the choice must be developed. Several questions must be addressed. What species of microorganism(s) should be used in the challenge? Should vegetative cells or spores be used? Should the aerosol of microorganisms be generated from a microbial suspension in liquid or particles contaminated with microbes? If a liquid aerosol is used, what solvent should be used along with it? What size particles or droplets should be used?

A second area of concern to the investigator is to ensure that the microbial challenge each package receives is defined and proven to be reproducible. Again, more questions. How many microorganisms should be used to challenge the package? Should the challenge be based upon the number of microbes per package or normalized to the surface area of the package? Should the package be challenged in different orientations? What is the best way to demonstrate the reproducibility of the challenge? When the package size, shape, or component materials change, do the test conditions have to be changed?

Third, the exterior of the package should be disinfected to reduce the probability of obtaining a false positive during sterility testing. What is an appropriate level of disinfection? Should a physical or chemical disinfection process be used? If a physical method such as ultraviolet (UV) light is chosen, are the packaging materials UV transparent? The use of UV light may actually reduce the microbial population inside the package if the film component allows UV transmission. If a chemical disinfectant is chosen, does the liquid permeate the porous packaging material and does the material have sufficient wet strength to withstand chemical disinfection?

Fourth, microorganisms that have entered the package must be recovered and cultured. If the surface area of the device is relatively small compared to the surface area inside the package, should additional techniques be used to improve recovery of microbes that have entered the package? These may include swabbing the interior package surfaces, direct culturing by aseptically placing the growth medium inside the package, and the use of dunnage.

After all of these variables (and others not discussed here) are taken into account, the chances of establishing a single, properly validated test procedure applicable to a wide range of package designs and packaging materials would be very small. Industrywide, the inevitable result is a multitude of customized test protocols that may have only a few traits in common, namely, that packages are sprayed with microorganisms and the contents are tested for sterility. But even if a methodology could be properly validated for just one package, the fundamental question associated with sterility testing as a method of assessing sterility maintenance still remains. What do the results mean?

Evaluating Challenge Results. If the contents of sterilized packages do not demonstrate microbial growth after sterility testing, one interpretation can be that the package has maintained sterility and is therefore acceptable. An alternative interpretation is that the sterility test may not be sensitive enough to demonstrate whether package integrity has been maintained. If a package has a breach in its integrity, it is still possible for the contents to remain sterile. Pasteur proved this during the nineteenth-century debate on spontaneous generation by using swan-neck flasks in which he had intentionally created a tortuous path that prevented contamination and maintained sterility. But a breach in package integrity (such as a channel in the heat seal) represents an unintentional manufacturing defect, and no company would deliberately set out to validate and commercialize a device whose package included such defects. (Imagine the labeling, "Sterility guaranteed even if channels are present in the package seal.") Since the goal is to manufacture packages without defects, it would be impractical to validate the ability of packages with defects to maintain sterility.

When a positive sterility test is obtained, what does it mean? Did the package truly fail and allow ingress of microbes? Was the package failure due to a design flaw or a material flaw? Were the package seals intact and continuous? Was the package intact but the technician made an error? Sterility testing alone does not answer these questions. A packaging engineer would typically use visual examination and physical test methods to identify possible defects that could have resulted in loss of package integrity and, therefore, loss of sterility.[8]

As a result, several medical device manufacturers have followed a natural progression of thought to the point of asking themselves, "Why conduct sterility testing with stability and environmental challenge studies? Why not perform just microbial barrier testing for the porous package component, and use physical tests to determine package integrity?" Proponents of this approach have argued that if a device has been properly sterilized, if its porous packaging material is known to have acceptable barrier properties, and if the integrity of the package has not been breached, then the device should be presumed to be sterile.[1,11,12] This rationale is outlined in the proposed International Organization for Standardization (ISO) packaging standard (DIS 11607), which is currently being balloted for approval.

DESIGN OF THE HIMA STUDY

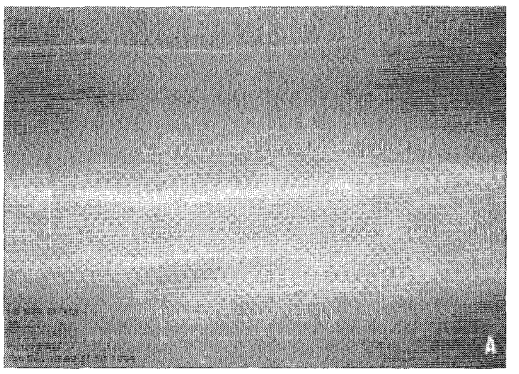


Figure 1. Seal of a film/film package prepared as a control sample (A) for the HIMA Sterile Packaging Working Group study.

The first task of the HIMA working group was to develop a protocol that would enable investigators to determine the relative abilities of whole-package microbial challenge testing and physical test methods to detect defects in the seals of various types of device packaging. Rather than attempting to optimize the sensitivity of a particular methodology or establish standardized test conditions, the protocol design focused on ways to develop data that would answer the question, "Is physical testing as sensitive as whole-package microbial challenge testing?" The protocol was submitted to FDA for agency review and comment, and the study was initiated once technical differences over the protocol design had been resolved.

The protocol was divided into two parts, a preliminary study and a full study. The preliminary study was designed to be a range-finding exercise to determine the appropriate defect sizes that should be used in the full study. Consequently, it used only one packaging configuration (film/film pouches). The full study was designed to investigate other packaging configurations including rigid and flexible packaging as well as porous and nonporous materials. In addition, the design of the full study called for the evaluation of physical test methods beyond visual inspection and dye penetration, including particle transmission and pressure differential testing.

Hypothesis. The null hypothesis adopted for the study was that the sensitivities of physical and microbial test methods in detecting package seal defects do not differ significantly.

Investigation. The objective of the study was to evaluate the relative abilities of two commonly used physical test methods and one microbial challenge test method to detect channels in the seals of nonporous whole packages. In the design of the study, the following assumptions about routine packaging processes were made:

- The materials used in manufacturing the final package have been evaluated and are appropriate.
- The package design has been qualified and is appropriate for the intended use.
- The packaging process has been validated and is in a state of control.

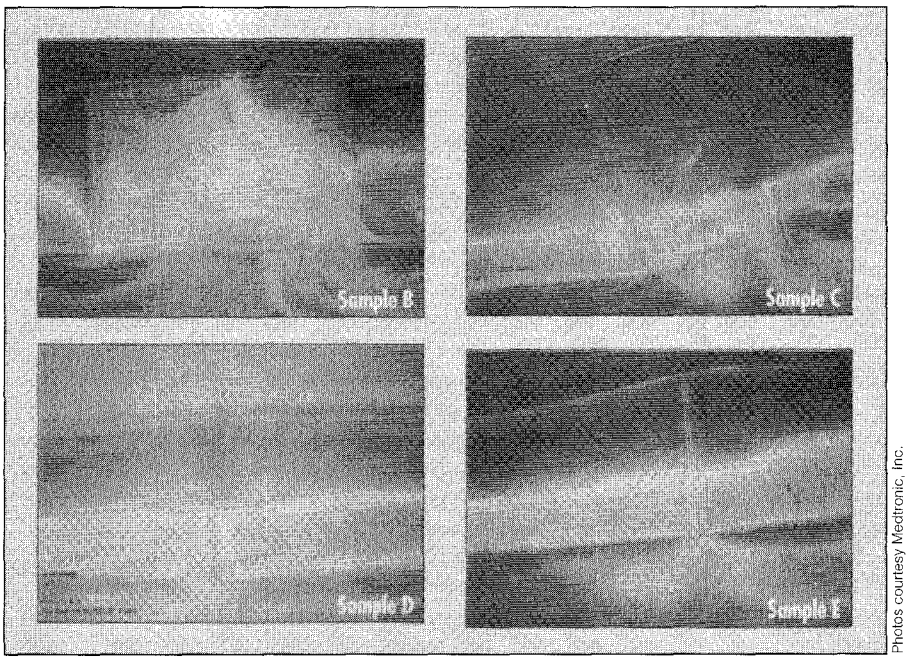
With these assumptions in place, the only area in question regarding the integrity of the whole package is the seal, which was therefore the entire focus of the study.

Preparation of the Samples. Film/film packages were formed using a Sentinel bar sealer, Model 12AS, with a ⅜-in. sealing bar. The package film was a 5½ × 8½-in. chevron pouch (polyester/polyethylene) with ⅜-in.-wide seals, provided by Tolas Health Care Packaging (Feasterville, PA).

Contents of the packages were Scott paper toweling folded in half to 3¾ × 5⅛ in. and #16000 light blue round plastic forceps by Qosina Corp. (Edgewood, NY). These products were chosen both to simulate a packaged medical device and to fill a large surface area within the package for capturing microorganisms that might penetrate it during the microbial challenge procedure.

The sealing process was characterized by sealing 20 packages at each of the upper and lower process conditions (250° ± 5°F). All packages were inspected visually for continuous and homogeneous seal appearance (see Figure 1). Instron seal strength evaluations were performed with the following average seal strength results: seals produced at 245°F yielded an average strength of 1081.7 g, and seals produced at 255°F had an average strength of 1142.5 g.

Defective packages were made using shims and wires ranging in size from 0.375 to 0.005 in. to produce channels in the package seals (see Table I and Figure 2). Each defect was placed in an area of the package that would allow it to be opened without disturbing the defective portion of the seal. Prior to testing, all packages were gamma irradiated to achieve a minimum dose of 25 kGy.



Photos courtesy Medtronic, Inc.

Figure 2. Sample packages with intentional seal defects produced by a ⅜ × 0.005-in. shim (B); a 3/16 × 0.005-in. shim (C); a 0.010-in.-diam wire (D); and a 0.005-in.-diam wire (E).

WHOLE-PACKAGE MICROBIAL CHALLENGE TEST

Thirty packages of each type were subjected to whole-package microbial challenge testing. The testing began with samples that incorporated the largest seal defects, and continued to evaluate progressively smaller defects until they became undetectable by either the microbial challenge test or the physical test methods. Care was taken to open the packages without disturbing the defective seal, and the packages were retained for evaluation by dye penetration, visual inspection, and seal characterization testing.

Sample Designation	No. of Samples Produced	Defect-Producing Device	
		Type	Size (in.)
A	30	None (control)	—
B	45	Metal shim	3/8 × 6 × 0.005
C	45	Metal shim	3/16 × 6 × 0.005
D	45	Wire	0.010 diam × 6
E	45	Wire	0.005 diam × 6

Table I. Sample package preparation.

The chamber used in the study was identical to the chamber described by Reich, with the exception that it was slightly larger (see box).[10,13] The chamber measured 22 (H) × 24 (W) × 20 (D) in., and was constructed of ¼-in. Plexiglas. The chamber was fitted with sample-suspending bars and clamps, three impinger sampling ports, a circulating fan, and a nebulizer. The Dayton Electric Manufacturing Co. (Chicago) circulating fan had a variable speed to provide air circulation from 25 to 200 cu ft/min, and it could be rotated 360° within the chamber. The chamber's nebulizer by DeVilbiss Health Care, Inc. (Somerset, PA), was a reflex atomizer; that is, the impacted fluid was returned to the reservoir and reatomized. Larger particles tended to be impacted and removed from the aerosol. Previous work has shown that this nebulizer consistently produces uniform-sized particles in the range of 0.3 to 2.0 µm, with a few particles above 2.0 µm.[14,15] The microbial challenge test was performed under ambient conditions for temperature, relative humidity, and pressure. The air pressure to the nebulizer was controlled by a U.S. Gauge air regulator.

The organism chosen for the microbial challenge test was *Bacillus subtilis* var. *niger* spores (ATCC #9372). The spores were grown at 30°–35°C, harvested, washed, and resuspended.[16] The spore suspension was then refrigerated at 2°–8°C until use.

The chamber was qualified by performing a microbial distribution assessment. Replicate organism detection and counting (RODAC) plates filled with soybean-casein digest agar (SCDA) were distributed throughout a fully loaded chamber. A spore suspension was then delivered to the chamber in the same manner as that to be used for the microbial challenge test, and the RODAC plates were incubated at 30°–35°C for approximately 24 hours. After incubation, the plates were evaluated to ensure that homogeneous microbial distribution had been obtained.

The microbial challenge test was performed by injecting 0.4 ml of 1×10^6/ml *B. subtilis* spores into the nebulizer to obtain a theoretical challenge of 4×10^5 per 0.2 m^3 chamber volume.[17] Each exposure contained a set of samples that consisted of 15 packages containing a defect and 4 packages without a defect (i.e., negative controls). Each set of samples was exposed for 30 minutes with a nebulizer airflow driving pressure of 5.0 psig and a fan velocity of 25 cu ft/min. Following exposure, the packages were removed from the chamber, wiped down with 70% isopropyl alcohol to sanitize their exterior surfaces, and allowed to dry under a Class 100 ventilation hood. Although UV light is often used to disinfect package surfaces after exposure to a microbial challenge, that was inappropriate in this case because the transparent film/film package would allow UV transmission that could inactivate any microbes that had penetrated the package.

A test of sterility was performed by aseptically placing the paper toweling and plastic forceps from each package into 500 ml of soybean-casein digest broth (SCDB), one container for the toweling and one for the forceps, and incubating at 30°–35°C for 7 days. For the packages containing an intended 0.010-in. defect (sample designation D) and the corresponding negative controls, both the towel and forceps were tested in a single container of SCDB, and incubated at 30°–35°C for 7 days (see Table II). In addition to the product negative control, the following control tests were also performed.

Bacteriostasis. Following the sterility test's 7-day incubation period, an SCDB container that displayed no growth was inoculated with less than 100 spores of *B. subtilis*. The container was incubated at 30°–35°C and growth of the indicator organism was observed within 3 days.

Package Surface Quantitation. Following exposure to the test conditions, a 4 × 4-cm section of the positive control package was excised and overlaid with approximately 30 ml of SCDA for each set of samples tested. Results of this test indicated that a range of 1.1×10^3 to 4.6×10^3 indicator organisms were deposited on the surface of the package.

Aerosol Quantitation. During the exposure, a 100-cc aliquot of air was taken from each of three locations in the chamber for each set

THE WHOLE-PACKAGE CHALLENGE

Ethox Corp. (Buffalo, NY) has been conducting microbial challenge tests to confirm whole-package integrity since 1988. The company's method has been the same as that described in the adjacent article, using an aerosol suspension of *Bacillus subtilis* var. *niger* in an enclosed chamber to create a microbial challenge, and then conducting sterility testing to evaluate the effectiveness of the device packaging.

The samples tested by the company represent a wide range of packaging options, including porous and nonporous pouches, lidded trays, and double-sterility-barrier combinations such as lidded trays within pouches.

Since initiating the procedure seven years ago, Ethox has tested approximately 7500 package samples. Using the company's whole-package microbial test method, *B. subtilis* has been recovered from fewer than 75 (less than 1%) of these test samples. However, visual examinations conducted prior to the microbial challenge testing have detected breaches in the packaging of all but 11 (approximately 85%) of those suspect samples.

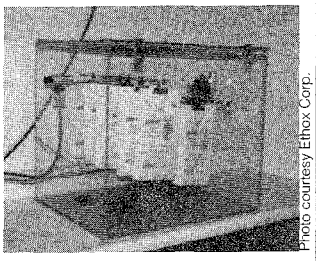


Photo courtesy Ethox Corp.

The microbial challenge test chamber used for the HIMA working group study.

The 11 positive samples for which visual examinations could not confirm a breach in the package seal represent a ratio of 1 in every 750 packages resulting in a positive sterility test. This ratio is consistent with industry rates for contamination when conducting sterility testing on products. It is sometimes referred to as the *false positive rate*.

Sample Designation	No. Samples Indicating Growth (per 30 Tested)	
	B. subtilis	Other Organisms
A	1[a]	1 towel
B	1 towel	1 towel & 1 forceps
C	0	1 towel
D	1[b]	1[b]
E	Not tested[c]	Not tested[c]

[a] The test of sterility for samples designated A (which were tested as controls concurrently with Sample D) was performed by testing the towel and forceps together.
[b] The test of sterility for samples designated D was performed by testing the towel and forceps together. Therefore, the product containing the organism could not be identified.
[c] Sample E, with the smallest channel size, was not tested in the whole-package microbial challenge because the larger channels formed in other samples had not been detected with consistency and reliability.

Table II. Microbial challenge test results.

Sample Designation	No. Samples Tested	Avg. Channel Size (in.)	Maximum Time for Dye to Penetrate All Samples
A	30	Control	No penetration after 1 hour
B	30	0.439	<5 seconds
C	30	0.236	<15 seconds
D	30	0.052	<5 seconds
E	30	0.022	<20 seconds

Table III. Dye penetration test results.

Sample Designation	Avg. Channel Width (in.)	Range (in.)
A	None	None
B	0.439	0.406–0.500
C	0.236	0.211–0.258
D	0.052	0.035–0.071
E	0.022	0.018–0.031

Table IV. Open-channel defect characterization.

of samples tested. Each aliquot of air was drawn from the chamber through a sterile 0.45-µm bacterial retentive membrane. The membranes were then transferred to petri dishes containing SCDA and incubated at 30°–35°C for approximately 24 hours. Results of this test indicated that a range of from 5×10^4 to 5×10^5 indicator organisms were available in the chamber during the test.

PHYSICAL TESTING

The physical testing methods used in the preliminary study included dye penetration testing and visual inspection of the package seals, which were conducted independently by Donbar Industries, Inc. (Long Valley, NJ). In addition, the seal defects were evaluated to determine their uniformity across the seal.

Dye Penetration Inspection. Thirty packages of each type that had already been used for whole-package microbial challenge testing were also subjected to a dye penetration test. During the earlier testing, the packages had been opened to within approximately 1 in. of the seal defect.

In this test, 15 cc of methylene blue solution (0.01% by weight) was placed into each package. The packages were then held vertically and the time required for the dye to penetrate the package defect was recorded (see Table III).

Visual Seal Inspection. Visual inspection of the package seals was conducted, without use of magnification, with packages held approximately 12 to 18 in. from the observer. The time required for each observation was less than 8 seconds. All of the samples that had been produced with intentional channels (samples designated B–E) were visually examined and their channels were detected. The samples that were produced without intentional defects (samples designated A) were visually examined and no channels were detected.

Open-Channel Defect Characterization. To determine the uniformity of the channel defects that had been intentionally created during package production, the defects were measured and evaluated. The samples used for this purpose had undergone the same handling and shipping—from manufacturer to sterilization facility to testing laboratory—as had the samples used for the whole-package microbial challenge testing. The testing was performed after shipping in order to be certain that the channels had not resealed while they were in transit.

Although it was originally intended that measurements of the seal defects would be made on the outer edge of unopened packages, backlighting proved insufficient to permit this technique to be used. Instead, the size of the channels was recorded by peeling apart the package and measuring the channel at its outside edge. All samples were viewed under magnification (10× for samples designated B, C, and D, and 50× for samples designated E) and photographs of representative samples were taken to show channel uniformity (see Figures 1 and 2).

Channels created using metal shims (samples designated B and C) were relatively uniform across the seal width, and an average channel width was calculated based upon measurements of the inner and outer edges of the channel openings. It should be noted that with these samples, the narrowest point of the channel was at the outer edge and the widest point was at the inner edge.

Channels created using wires (samples designated D and E) were narrow at the inner and outer openings while the middle of the channel generally had a wider, bowed appearance (see Figure 2). Therefore, measurements were taken and averaged at the outer seal opening and at the narrowest and widest points (see Table IV). It should be noted that the channels of many of the samples designated E appeared to be clogged or blocked with adhesive, which may have occurred during preparation of the channel.

CONCLUSION

The results reported in this article are those obtained from the preliminary study designed by HIMA's working group. Of the 150 samples examined for the study—30 control samples and 120 with intentional seal defects—the whole-package microbial challenge test resulted in correctly positive findings of the test organism in only two instances. Equally important, the microbial challenge test also resulted in a false positive in one control sample, and positive findings of organisms other than *B. subtilis* in four instances. Meanwhile, the physical testing methods used for this study were able to correctly identify all 90 of the samples containing seal defects, with no false positives for samples without defects.

On the basis of these findings, the null hypothesis of this investigation must be considered invalid: There are significant differences between physical and microbial test methods in detecting package seal channel defects. In this study, the microbial challenge test did not consistently and reliably detect even the largest package channel defect, while visual inspection and dye penetration tests were each sensitive enough to detect the smallest package channel defect produced in all packages. Earlier work by Spitzley also supports this conclusion.[8]

Although this study was originally intended as merely preliminary to a larger study that would consider the effectiveness of the testing methods for a wider range of materials and packaging configurations, it is clear that an expanded study would not add to the general picture developed here. The consensus of the HIMA Sterile Packaging Working Group is that physical tests for evaluating whole-package integrity are more effective than whole-package microbial challenge testing for the following reasons:

- The inability of the microbial challenge method to consistently detect package seal defects of the sizes tested.
- The occurrence of microbial contamination (other than the challenge organism) in the samples used for this study.
- The occurrence of microbial contamination in some of the control packages (packages with no defects).

Since this evidence demonstrates that whole-package microbial challenge testing may not reliably detect channels in whole packages, the working group has concluded that the test should not be used for that purpose unless properly validated (i.e., through a demonstration of repeatability and reliability) for each package configuration. Although physical tests must also be validated, that validation may be applicable to a broader range of packages and package types and may not require a customized test procedure for each package configuration. Physical test methods such as the dye penetration and visual tests examined in this study can consistently and reliably detect seal channels as small as 0.018 in., and are especially useful for transparent materials such as the film/film packages evaluated here. These results support the claim of physical testing to be considered an alternative methodology for assessing intact package integrity.

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IN QUEST OF STERILE PACKAGING: PART 2

PHYSICAL PACKAGE INTEGRITY TEST METHODS

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For more than a decade, the approaches and methods used by device manufacturers to test the ability of a medical package to maintain sterility have been subjects of considerable debate. As reported in the first installment of this article (*MD&DI*, August 1995), the Health Industry Manufacturers Association (HIMA) Sterile Packaging Working Group recently undertook an investigation designed to resolve some of the questions related to the testing of sterility maintenance in medical packaging.[1]

The HIMA study compared the results of a whole-package microbial challenge test versus visual inspection and dye penetration tests on film/film packages formed with intentional seal channel defects. The study demonstrated that dye penetration testing on transparent film/film packages can reliably and consistently detect seal channels as small as 0.018 in. The study also supported the use of visual inspection for detecting channel defects of the smallest size tested.

On the other hand, the working group's comparisons indicated strongly that a whole-package microbial challenge test followed by sterility testing may not reliably detect seal channels in whole packages. As a result, the group concluded that such testing should not be used for that purpose unless the method is properly validated (i.e., through a demonstration of repeatability and reliability) for each package configuration. Earlier work by Spitzley supports this conclusion.[2]

The question still remains regarding which physical test is appropriate to demonstrate whole-package integrity for a given package. This installment of the article provides guidance on the selection, use, and limitations of physical package integrity test methods based on the material and package type. It is not meant to be an exhaustive treatment of all methodologies, but rather represents a practical approach for choosing an appropriate method.

Many times manufacturers use test methods without validation simply because the tests have been published in a recognized compendium or have been accepted as industry standards. A single test method may not be appropriate for all situations, materials, or package designs. During the package design phase, for example, a discriminating quantitative test may be desirable, whereas a simpler go/no-go integrity test may be more appropriate for routine in-process checks of a validated process.

A selected test method should include a documented rationale along with validation data that address its sensitivity, reliability, and repeatability.

A selected test method should include a documented rationale along with validation data that address its sensitivity, reliability, and repeatability. The method should be consistent with the appropriate sensitivity level relevant to a product's requirements. A test method's sensitivity compared to whole-package microbial challenge testing is significant, especially if the test is used to establish sterility maintenance; that is, the package's ability to maintain sterile integrity over time.

Typically, sterile medical device packaging materials can be divided into two categories: impermeable (nonporous) and porous.

Impermeable materials such as film or foil provide product-protecting barriers to moisture, light, and air, and can be sterilized by radiation or dry heat. Generally, integrity testing of impermeable packages evaluates both the material and the packaging seals. A pressure differential or flow can be easily created across the barrier to measure leakage. Porous materials such as sterilizable papers and spunbonded polyolefin allow air to pass, making them compatible with sterilization methods such as gas and steam. Integrity evaluation of porous packages presents a greater challenge, and therefore provides more reason to determine the test sensitivity for a particular material and package construction. Whole-package integrity test methods for porous materials tend to evaluate the integrity of the seal only, and thus depend on other means to qualify the microbial barrier properties of the material.[3–5]

This article distinguishes between impermeable and porous materials for each test method described. The recently published American Society for Testing and Materials' (ASTM) standard F 1585 *Guide for Integrity Testing of Porous Barrier Medical Packages,* provides guidance for selecting appropriate physical test methods.[6]

Package designs can typically be classified as rigid (blisters and trays) or flexible (pouches). For each combination of material and package design, manufacturers must define failure and challenge the package with a method that demonstrates that failure. Choosing an appropriate cost-effective test method often depends on the nature of a given package and its contents and on whether the test method is destructive. Table I lists the whole-package integrity test methods discussed in this article.

TRACE GAS SENSING

Trace gas sensing tests flush or pressurize the inside of the package with a gas such as nitrogen, helium, argon, or oxygen, and then use leak-detection sensors to identify voids, cracks, tears, channels, or pinholes. Selecting the proper trace gas depends on the package design, packaging materials, and package contents. These tests are generally nondestructive and are particularly suited for ongoing, online testing.

Test Method	*Material Type*	*Equipment Cost*	*Destructive*	*Complexity*	*Reference*
Trace gas sensing	Impermeable Transparent/ opaque	High	No	Complex	ASTM E 1316 ASTM E 43 ASTM E 47
Pressure differential					
Bubble immersion	Impermeable Porous limited	Moderate	Yes	Moderate	ASTM D 3078 ASTM D 4991
Vacuum dye	Transparent/ opaque				ASTM E 515
Pressure decay	Impermeable Transparent/ opaque	High	No	Complex	TBD
Visual inspection	All types	Low	Yes and no	Simple	In progress
Light transmission	Impermeable Porous Opaque	Low	No	Simple	No
Dye penetration	Impermeable Porous limited Transparent/ Opaque limited	Low	Yes	Simple	In progress
Particulate transmission	All types	Not commercially available	Yes	Moderate	No

Table I. Physical test methods for package integrity.

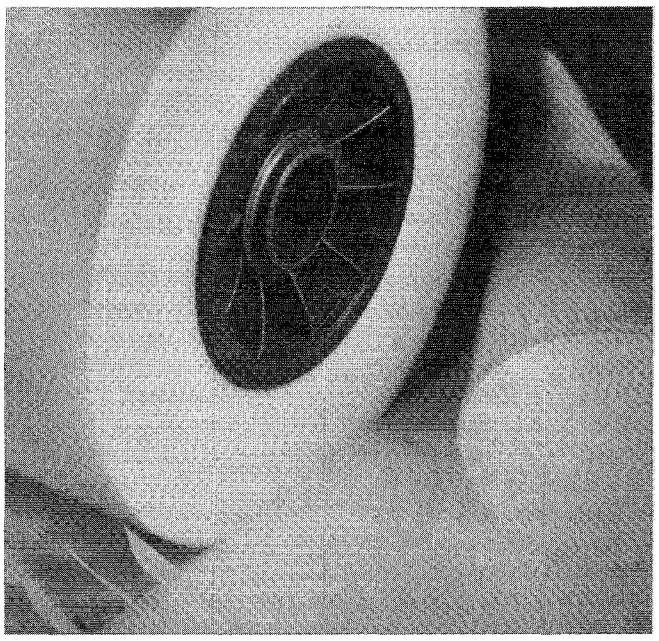
Some important considerations must be addressed before using this test method. The most significant is whether the package is impermeable or porous. Trace gas testing is most commonly applied to impermeable packages. Because the package materials are impermeable to air, any breach in the materials or seals is detected quickly when the gas flows through it. Besides detecting a breach in the package, the test can usually locate the defect, allowing the manufacturer to identify the anomaly more easily and take corrective action.

Trace gas is difficult to apply to porous packages. When packages are sterilized using steam or a gaseous sterilant, the packaging materials must allow the exchange of air to facilitate the ingress and outflow of the steam or sterilant. Trace gases can pass easily through the structure of porous materials. Therefore,

trace gas sensing is an improper test for package integrity unless a manufacturer compensates for the material's porous structure.

One way to accomplish this is to mask the surface of the porous material to make it impermeable. Another way is to seal off the porous surface with a gasketed fixturing. These adaptations, in effect, isolate the porous materials and concentrate the test on the seals and the package's impermeable surfaces.

Trace gas testing is considered the most sensitive of integrity tests, including many microbial challenge tests.[7] Manufacturers must be judicious in establishing test sensitivity levels. Trace gas detection can locate microscopic holes and channels that might not, in fact, impair sterile package integrity. Users need to consider the possibility that overly stringent sensitivity levels may reject packages normally considered effective in maintaining sterile integrity.



PRESSURE DIFFERENTIAL

Manufacturers can evaluate package integrity by creating a pressure difference across the barrier and examining the test sample for changes. Tests include bubble immersion, vacuum dye, and pressure decay.

Bubble Immersion Testing. A common way to perform a bubble immersion test is to submerge test samples in a liquid-filled sealed vacuum vessel, drawing a vacuum inside the vessel and observing the sample for leaks indicated by escaping air bubbles. Several standardized methods are available to perform this type of test, including ASTM D 3078, *Standard Test Method for Leaks in Heat-Sealed Flexible Packages;* D 4991, *Standard Test Method for Leakage Testing of Empty Containers by Vacuum Method;* and E 515, *Standard Test Method for Leaks Using Bubble Emission Techniques.*[8–10]

Vacuum Dye Testing. Adding a dye to the liquid in the vacuum vessel changes the above method to a vacuum dye test. In this case, if a hole or channel exists, the dye penetrates the package when the vacuum is released. This test is particularly appropriate for transparent packages, because the dye is visible inside. Studies have demonstrated that using these tests to detect dye solutions in transparent packages is as sensitive as spectrophotometric methods, 1×10^{-3} mg/ml.[11]

There are several factors to consider when choosing these pressure differential tests. Again, the most important is whether the package is impermeable or porous. As discussed in ASTM F 1585, *Guide for Integrity Testing of Porous Barrier Medical Packages,* mentioned earlier, manufacturers must use caution with these methods because they were not developed for use with, nor do they specifically address how to handle, porous materials.[6] Erroneous results can be reported if an operator is unfamiliar with their limitations.

Overly stringent sensitivity levels may reject packages normally considered effective in maintaining sterile integrity.

The most important material characteristic to determine when performing pressure differential tests on porous packages is the bubble point of the most porous material in the package. A package containing a porous material immersed in a liquid reaches its bubble point when the air or gas on one side of the porous material overcomes the forces restricting its flow through that material, and a bubble forms on the opposite side. The same effect occurs with pressure. The key is to determine the vacuum or pressure parameter that does not exceed the bubble point of the material. If the bubble point is exceeded, bubbles form or liquid penetrates the pores of the material, erroneously indicating a leak.

The porosity of a packaging material may cause a variation in the bubble point from sample to sample and within the same sample. For instance, porous materials with a heat-seal coating can exhibit porosity value ranging from 30 to 300 gurley seconds. This makes it difficult for a test operator to use a set bubble point for all samples of the same package design and material. Also, because porosity varies, materials with low bubble points limit the maximum pressure differential. This in turn limits the sensitivity of the test relative to the channel size it can detect.

Additional factors that may produce erroneous test results include the package contents, the nature of the paper, and the softening of heat-seal coatings. Contents in contact with the package material may plug a leak. As stated in the scope of ASTM D 3078, "Small leaks may not be detected. Viscoelastic effects on the products or entrapped air become significant and prevent passage through small leaks."[8] Paper materials can rapidly deteriorate when submerged in a liquid and can cause leaks to swell and close. Weakened paper also may exhibit premature bubble emission or liquid penetration. Lastly, using a heated test fluid as specified in ASTM E 515 to create a pressure differential softens the heat-seal coatings on package materials and seals the leaks.

To counteract the effects of porous materials, manufacturers have tried a variety of sample preparation techniques. These techniques involve masking the material by using pressure-sensitive label stock, petroleum jelly, varnish, or pressure-sensitive tape, minimizing the porous nature of the material and focusing on seal integrity.

Pressure Decay Testing. Pressure decay testing is another effective method for examining impermeable packages for leaks. This test incorporates specially designed equipment that creates a pressure differential across the barrier and evaluates any material movement or change in sample pressure that indicates deflation or leak. Variables such as the contents and variations in head space can affect the amount of flexure in the package.

Each test setup should address the specific package configuration being tested. Custom fixtures are often designed for specific package configurations. When attempting to use this method on porous packages, sensitivity becomes critical because the variations inherent in porous materials limit the reproducibility and sensitivity of the test.

Pressure-differential test methods can be effective tools for impermeable package integrity testing. Their application on porous barrier packages can also be effective; however, users must compensate for the nature of these materials and adapt test methods appropriately.

VISUAL SEAL INSPECTION

Package seals, typically accomplished with heat, are defined as two or more surfaces united by fusion of either the coatings or the base material under controlled temperature, pressure, and time (dwell). Packages constructed of at least one transparent component allow the seal to be visible. In some applications, certain heat-seal coatings fluoresce in the presence of ultraviolet (UV) light of a certain wavelength.[12] If the seal is visible through the package, it can be examined nondestructively under a UV lamp.

Other materials and heat-seal coatings show a distinctly different color or contrast in the sealed and unsealed areas. Some translucent films show a clear seal area when heat-sealed, whereas other materials have tinted seal coatings. If some of the sealant is missing or if a seal is inadequately formed, the package will appear different in that area.

Manufacturers can perform a series of destructive tests to evaluate package integrity by visually examining the seal after peeling the package open. This is particularly appropriate for opaque materials. Sealant on peelable packaging generally leaves a highly visible residue or transfers to the facing surface after the package is opened. Theoretically, the bond on the opposite sealing surface is stronger than the original substrate. To indicate seal integrity, this residue or transfer should be uniform and continuous. Incomplete seal transfer can indicate a flaw in the seal itself or in the sealing process. Seal attributes such as spottiness, uneven seal width, voids, and channels may then be characterized depending on severity level.

Heat seals should be cooled to ambient conditions before peeling apart to allow the adhesive to bond to the opposite substrate. Opening the package too soon may result in incomplete adhesive transfer. A smooth continuous peeling motion should minimize extraneous defects. A jerky peeling motion could result in a defective appearance. For example, adhesion characteristics of the base materials may cause peeled packaging to have a channel or void across the seal that did not exist in the intact, unopened state. This may be interpreted as a package integrity failure. In such instances, a more sensitive package integrity test may be required to confirm whether the channel or void is in fact an unsealed area.

The visual seal inspection test is easy and inexpensive to run and has been widely used in the medical device industry. Magnification is sometimes used to enhance visibility of the inspected areas. Automated vision systems can evaluate seal attributes either on- or off-line. This method should be correlated with an accepted, validated physical integrity test with known or demonstrated sensitivity. Currently, no standardized method or practice exists; however, the ASTM F 2.6 Medical Packaging Subcommittee recently drafted a test method to standardize the procedure.

LIGHT TRANSMISSION

Light transmission is used frequently for pinhole detection. Although this method is typically used with aluminum foil structures, it could be applied to any package with one opaque side. The package is placed over an intense light source with the opaque side toward the light, revealing any holes in the material. Inspection in a darkened room is generally recommended.

When using composite materials, inspectors must determine whether the pinhole penetrates the entire structure or only the opaque layer. For instance, a light transmission test may reveal pinholes in laminated foil and clear film structures, but if pinholes are revealed only in the foil and not in the clear film, sterile integrity would still exist. A light transmission test is typically not used with porous materials because light easily passes through most of them.

DYE PENETRATION

Dye penetration testing is useful for detecting defects such as holes, channels, and voids. This test injects a package with a dye solution, exposing the package for a specified time. The package is then inspected, particularly around the seal. The solution normally consists of distilled water, a dye agent (such as methylene blue), and a surfactant to reduce the surface tension of the water and facilitate penetration of the solution into and through small holes, cracks, and channels.

Testing has demonstrated that dye penetration evaluations for package integrity are more sensitive than a whole-package microbial challenge test.[1,2] Dye penetration testing is destructive because the package must be penetrated to inject the dye solution. Therefore, it is less than ideal for on-line integrity testing.

Gravity is usually sufficient to force the dye solution into any potential package defect areas. Small packages, packages with few internal cavities, or packages with devices that occupy all or most of the internal space must be considered carefully before selecting a dye penetration test to evaluate integrity. This ensures that an adequate amount of solution can be injected for effective capillary action. According to Joe Spitz, vice president for technology development at Rexam Medical Packaging (Vernon Hills, IL), "If the surface tension of the solution is greater than the capillary forces drawing the solution through the defect, penetration of the dye solution will not take place."

If a dye penetration test is used to evaluate package seals, it is helpful for one of the substrates to be transparent so that potential seal defects can be observed easily. Dye penetration can also be used to evaluate opaque package seals, but the test is more difficult to perform and evaluate because the evidence of dye penetration must be observed at the seal's outside edge. The amount of dye that would penetrate a void across a seal is very small and sometimes requires magnification to detect.

Dye penetration testing can also be used as a tool to confirm visible defects in package seals. Experiments conducted by the HIMA Sterile Packaging Working Group and by Spitzley included a visual examination for seal defects and the dye penetration test.[1,2] Dye penetration testing confirmed defects detected visually.

Dye penetration testing can be applied easily to impermeable packages because materials used in them are usually impervious to liquids. Both the seals and the package materials are readily evaluated by dye testing. The exposure time is limited only by the test sensitivity level required.

Several considerations must be made before using dye penetration testing on permeable packages. The dye solution can destroy the structure of some papers, which could eliminate dye penetration as a test candidate. When applying dye penetration tests to porous materials, the materials should be exposed to the dye solution for a limited time, particularly if the solution contains a surfactant. The flow of the dye through such materials can occur after a short exposure time.

The structure of porous materials consists of interwoven fibers

calendered or bonded into a continuous sheet. This fibrous structure can allow a solution, particularly one with a surfactant, to follow fibers from one side of the sheet to the other. This is called *wicking*. The occurrence of wicking does not preclude the materials from providing an effective microbial barrier. It could, however, make it difficult to determine whether the presence of the dye solution on the surface was caused by wicking or by a hole or path in the structure outside of the material specifications of the supplier. Therefore, dye testing for porous packages should be limited to evaluating seal integrity only.

PARTICULATE TRANSMISSION

Industry uses a number of test methods that incorporate the flow of particles across a pressure differential or that physically stress the package. Such tests may identify a hole in the material or a channel in the seal. The particles in an aerosol or suspension are generated inside or outside the test package. Manufacturers have used carbon black, talcum powder, powdered chalk, and size-controlled smoke particles as integrity tests for packages with tortuous path folds or crimps, such as paper bags.

Particulate transmission is one of the few physical test methods that may apply to this type of package. The main feature of this test is that the particulate readily locates the defect. However, some disadvantages should be considered, such as particles clogging the hole or channel, causing the defect to be sealed in the process. It is critical to control variables such as the flow rate, particulate concentration, and homogeneity of particle size.

VALIDATION OF TEST METHODS

When choosing a test method for evaluating sterile package integrity, a manufacturer must ensure that the test is appropriate for the package type and sensitive enough to detect possible compromises in the integrity of the sterile package. Several other considerations are important when selecting and implementing physical tests for sterile package integrity.

Documentation. Standardized test methods are documented, balloted, and published. Documentation provides details regarding performing the test and evaluating the results. Internally developed methods must be documented for consistency and reproducibility from operator to operator and among test sites. A rationale must be developed for applying the test method to a particular package configuration. Information must be provided regarding scope, equipment used, sample preparation, procedures, and reporting the data.

Reproducibility. Data must show that the test method can consistently locate known defects in the sterile barrier. This can only be done through replicate testing of statistically significant sample sizes containing packages with known defects.

Sensitivity. Test sensitivity to defect size should be established by producing and testing samples with defects of a known size. The size of the known defects should be reduced until the test method is no longer sensitive enough to detect the defect. In some cases, producing defects of a very small size (<0.003 in.) can be very difficult. This was shown in the preliminary study conducted by HIMA's Sterile Packaging Working Group, in which a known defect in a package seal created with a 0.005-in. wire was readily detected using dye penetration testing, whereas the microbial challenge method was unable to reliably detect the channels in the seals.[1] In this case, a test that was sensitive enough to locate such a defect would be a sufficient physical test method to replace the whole-package microbial challenge test.

CONCLUSION

Our review of the various test methods shows that the data support the hypothesis that certain physical test methods are more effective and consistent than present whole-package microbial challenge testing in locating channel-type defects that compromise the sterile integrity of a medical device package.[1,2] As mentioned, Table I summarizes some more commonly used physical tests, including their applicability, relative equipment costs, complexity, publication references, and whether the are destructive. Leak-rate sensitivity for impermeable packaging has been well documented. For porous packages, however, no documentation is available for sensitivity levels. Therefore, supporting documentation regarding test-sensitivity levels for porous packaging is essential.

Before selecting any test, it is important to remember that the most sensitive test may sometimes be inappropriate for a particular application. For example, a bubble immersion test may be more appropriate than a helium leak detection method for determining the sterile package integrity of a porous package. The type of package, the level of complexity, and whether the test is destructive are equally important considerations. The physical test chosen to assess sterile package integrity for a specific package configuration must be validated.

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