WHITE PAPER

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Medical Packaging Study –The Impact of Sterilization and Transportation Testing on the Microbial Barrier of Different Materials

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Overview

The principal function of packaging for terminally sterilized medical devices, also known as the sterile barrier system (SBS), is to maintain sterility by maintaining package integrity and microbial barrier, throughout all steps of the value chain, until the point of use in a healthcare setting.

The loss of package integrity can have serious consequences ranging from costly product recalls to compromised patient safety. A recent white paper titled: "Medical Packaging Study – Reducing the Risk of Failure through Performance Testing of Packaging Made from Various Materials" [1] explained the importance of conducting an established testing and validation process as crucial steps to ensuring package integrity and helping to prevent healthcare-associated infections (HAIs). The study discussed in that white paper, which was published in May 2014, emphasized that systematic environmental conditioning and transportation testing with subsequent package integrity testing will detect issues with packaging before the product is introduced to the market.

As follow up to that work, a second study was recently conducted. The purpose of this second study was to demonstrate the advantages of performing microbial barrier testing on porous materials in addition to package integrity testing post sterilization and subsequent environmental conditioning and transportation testing. This white paper summarizes the testing and conclusions of the second study.

Because common package integrity tests are limited in their leak detection sensitivity, microbial barrier testing will provide additional and more detailed input to assist in judging overall integrity and safety of a sterile package. This information can be very helpful because many manufacturers still rely only on the microbial barrier data provided by the material supplier.

The study discussed in this white paper provides an example of how a microbial barrier evaluation post transportation testing can be set up, and evaluates the performance of the different materials post sterilization, environmental conditioning and transportation testing.

Packaging used for this study were standard chevron pouches made with either DuPont™ Tyvek® 1073B, Tyvek® 2FS™ or one of four different commonly used medical-grade papers (see Table I), all having a comparable porosity performance (typical Gurley porosity better than 100 sec).

A total of 180 pouches were tested, representing six different material combinations. All pouches were subjected to microbial barrier testing before sterilization and after sterilization (ethylene oxide [EO] or Gamma) and environmental conditioning and transportation testing.

As shown by the data, a significant decrease in microbial barrier performance after Gamma sterilization and environmental conditioning and transportation testing compared to pre-sterilization has been reported for three of the four types of medical-grade paper that were evaluated in this study. This decrease in microbial barrier performance was mainly linked to creases and punctures in the material. The same three medical-grade paper types had the poorest overall microbial barrier performance post all environments, including pre-sterilization.

Two of the six pouch materials, Uncoated Tyvek® 1073B and Uncoated Tyvek® 2FS™, have shown the best barrier performance overall, compared to the four medical-grade paper types.

To assist readers who wish to further explore this topic, a list of test standards/references is provided.

Regulatory requirements

ISO 11607,[2-5] respectively EN ISO 11607,[6-9] Packaging for terminally sterilized medical devices, is a standard for sterile medical packaging recognized by the U.S. Food and Drug Administration (FDA) and harmonized with the essential requirements of the European medical device directives. The purpose of sterile barrier systems (SBS) is to maintain sterility until the point of use. A key concept is that maintenance of sterile barrier integrity may be used to demonstrate maintenance of sterility. In that sense, section 6.3.1 requires that "the integrity of the sterile barrier system shall be demonstrated after sterilization and subsequent performance testing". In section 6.3.2, under "Package-system performance testing," it is stated that "physical tests, along with *microbial barrier* testing of porous packaging materials, can be used to establish the capability of the sterile barrier system to maintain sterility." This standard also addresses *microbial barrier* in section 5.2.3, stating that "porous materials shall provide an adequate *microbial barrier* to microorganisms in order to provide integrity of the sterile barrier system and product safety." The standard (section 6.3.3) further states that "in the absence of applicable validated sterile barrier system integrity tests,[10, 11] *microbial barrier* performance requirements can be established by testing the *microbial barrier* properties of materials and the integrity of seals and closures."

Although the requirement to test seal integrity and microbial barrier is clearly defined in the standard, recent market feedback showed that many packaging applications have been validated with seal integrity testing only. Bubble leak testing evaluates the entire package integrity; however, the sensitivity is very limited[12] (ASTM F2096 indicates a sensitivity of about 250 µm, depending on test setup, that can be detected with a probability of 81%) and it cannot be considered a microbial barrier test method.

The ISO standard lists a number of test methods in Annex B (amendment from 2014[4, 8]) that can be used to perform package or material testing for qualification and validation purposes. There are several microbial barrier tests listed in Annex B, including ASTM F2638 "Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier," which has been selected for this study. Since January 2013, ASTM F2638 is also a recognized standard by the U.S. FDA.[13]

Scope of the study

For this study, the following material selection criteria were applied: focus on premium or the most commonly used medical-grade coated papers, all having a comparable porosity performance (typical Gurley porosity better than 100 sec). For DuPont™ Tyvek®, the medical packaging styles with the highest and lowest basis weight (Tyvek® 1073B and Tyvek® 2FS™) were selected.

A 2D chevron pouch was chosen as the package type in which a blood transfusion device was packed (see Figure 1).

Figure 1. Sample 2D chevron pouch filled with blood transfusion device.

The pouches were purchased from various sterile packaging manufacturers (SPMs) who offer the respective Tyvek® or medical-grade paper in combination with the appropriate film. See Table I for a list of materials used in this study.

Secondary packaging was defined by the contract packing service provider, the company responsible for the filling and sealing activities, as well as the organization of sterilization. The same transport packaging and sterilization conditions have been used for all pouch types.

Two types of sterilization were performed, ethylene oxide (EO) and Gamma (refer to Table II). Standard double cycles were applied according to the contract packing service provider.

The environmental conditioning, transportation simulation and microbial barrier testing were performed by independent accredited testing laboratories.

Table I. Materials Used in the Study

Material	Configuration
Uncoated DuPont™ Tyvek® 1073B	Pouch 260 (258) x 160 mm
Uncoated DuPont™ Tyvek® 2FS™	
60g/m² Paper / 11g/m² Grid Lacquer	
113g/m² Reinforced Paper / 13g/m² Allover Coating	
85g/m² Reinforced Paper / 11.5g/m² Allover Coating	
80g/m² Reinforced Paper / 3g/m² Allover Coating	
Bottom webs for DuPont™ Tyvek®	
PET-O/PE 12/50µm peel	
Bottom webs for medical-grade papers	
PET-O/PE 12/50µm or 60µm	
Shelf (inner) carton	521 x 365 x 187 mm
Transportation carton	548 x 394 x 438 mm
Transfusion set	N/A

Table II. Description of Sterilization Cycle

Sterilization Method	Target Dosage
Ethylene oxide (EO)	Two full cycles with pre-conditioning and aeration RH pre-conditioning: 60% ± 15% EO concentration: ~720 mg/L Temperature: 45 ± 5°C Cycle duration: ~12hrs Max. pressure rate change: 34 mbar/min.
Gamma radiation	Two cycles at min. 25 kGy (effective max. 36.1 kGy) each

Study summary—Microbial barrier testing post sterilization and environmental conditioning and transportation testing

A study protocol was established, outlining the test parameters required for the evaluation of the pouches.

Prior to environmental conditioning and transportation testing, the pouches were subjected to sterilization by either EO or Gamma radiation (double cycles). Unfilled, non-sterilized pouches were tested as reference.

Microbial barrier testing was performed by an independent accredited laboratory at the following conditions: pre-sterilization; post-sterilization and subsequent environmental conditioning and transportation testing.

As shown in Figure 2, packages were tested for integrity and strength in the first study, as described in the white paper titled: "Medical Packaging Study – Reducing the Risk of Failure through Performance Testing of Packaging Made from Various Materials."[1] In the first study, all samples were visually inspected and tested for seal strength and for seal integrity by both dye penetration and bubble leak tests, post different conditions. Within the second study, additional packages were subjected to microbial barrier testing. Testing performed within the scope of the second study is shown in red in Figure 2.

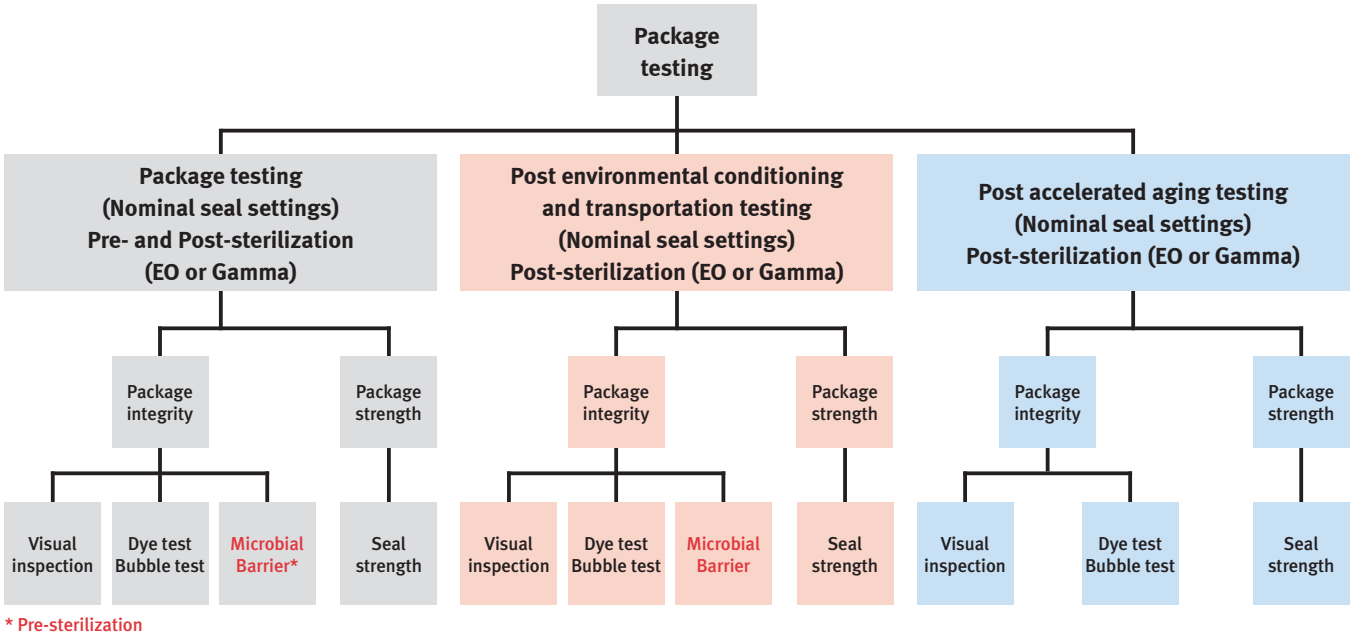

Figure 2. Process flow of testing—with tests conducted during second study shown in red.

Microbial barrier testing was performed according to ASTM F2638, "Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier.[13] This method measures the ability of a porous substrate to prevent particle penetration, which is highly correlated to microbiological spore penetration. The filtration efficiency of the material is evaluated using dual particle counters. The air flow through the samples is varied, using values comparable to real-world conditions, such as those found in air transport, handling, etc. The maximum penetration point is recorded in %. The lower the percent penetration, the better the performance.

This method allows defining the microbial barrier performance per material type and the assessment of the impact of specific environmental conditions on the microbial barrier performance. Data obtained from this test is useful in assessing the relative potential of a particular material to contribute to the maintenance of sterility of the contents of the package versus that of another porous material. See Table III and Figures 3-5 for test method details.

Table III. Defined Test Characteristics for Final Package Performance Evaluation

Test	Test Method	Test Details/Notes	Unit/Reporting	Acceptance Criteria	Sample Size per Pouch Type
Microbial barrier	ASTM F2638-12	Sample cutting (porous web only): Circle with ø 120 mm; Area with most creases selected, seal area not included Porous top web challenge side = outer pouch side Particle size 1 µm Particle challenge 3 MM/l Flow rate range 0.05—2 L/min	Maximum Penetration % (% pMax) Values reported individually Average/standard deviation calculated per environment Any visual anomalies observed in the porous material to be reported	The lower % pMax, the better the barrier performance Differences between materials and environments will be assessed	10 unfilled, non-sterilized pouches 10 filled pouches post environmental conditioning and transportation testing (EO) 10 filled pouches post environmental conditioning and transportation testing (Gamma)

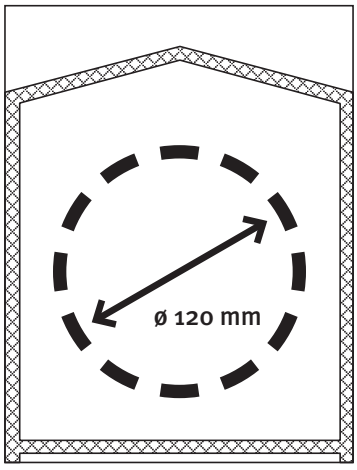

Figure 3. Microbial barrier sample cutting area on the pouch.

Figure 4. Microbial barrier test system (ASTM F2638).

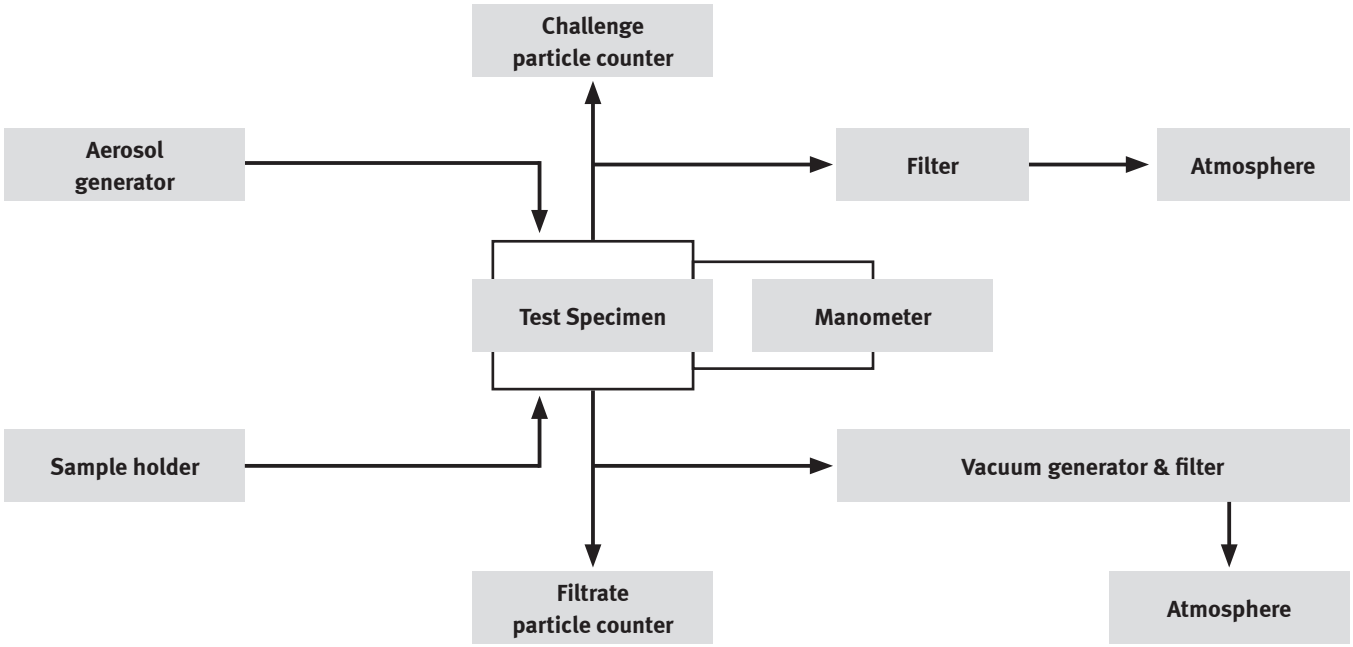

Figure 5. Typical schema of a microbial barrier test system (ASTM F2638).

Transportation testing

Prior to the transportation testing, the conditioning of the respective filled and sterilized pouches was carried out per ASTM D4332-01(2006) environmental conditioning for a total of seven days, as shown in Table IV (conditions partly adapted based on ASTM D4169, ISO 2233 and ISTA 2A as recommended by the independent accredited laboratory in order to reflect worldwide distribution worst case conditions).

Table IV. Pouch Environmental Conditioning

Anticipated Condition	Temperature (°C ± 2°C)	Relative Humidity (% RH)	Duration Time (hours)
Lab Ambient	Ambient	Ambient	6
Frozen or winter ambient	-35°C ± 2°C	—	72
Ambient	Ambient	Ambient	6
Tropical (Wet)	38°C ± 2°C	85% ± 5%	72
Desert (Dry)	60°C ± 2°C	30% ± 5%	6

After environmental conditioning, transportation testing was carried out in accordance with ASTM D4169-09 and associated international testing standards as described in Table V. Worldwide distribution with different means of transport has been assumed as a base for the selection of the test level and conditioning. The test has been based on Assurance Level I of ASTM D4169-09 Distribution Cycle 13 (DC-13).

Table V. Transportation Test Standards Sequence

Sequence	Test Schedule	Test Method / Standard
1	Conditioning	ASTM D4169, ASTM D4332
2	A: Manual Handling – First Sequence	ASTM D4169, ASTM D5276 / ISTA 2A*
3	C: Vehicle Stacking	ASTM D4169, ASTM D642
4	F: Loose Load Vibration	ASTM D4169, ASTM D999 Method A1
5	E: Vehicle Vibration – Truck and Air	ASTM D4169, ASTM D4728 Method A
6	A: Manual Handling – Second Sequence	ASTM D4169, ASTM D5276 / ISTA 2A*

* ISTA drop test heights were used with the ASTM drop test sequence.

Acceptance criteria prior to microbial barrier testing

Pouches were required to meet the following acceptance criteria before any further testing was conducted.

- Post conditioning:
  - The external shipper box shall not show any signs of deterioration after pre-conditioning. The closing tape must remain in position in all locations of the shipper.
- Post transportation:
  - Some slight damage to the sides and corners of the shipper box is allowed; however, the external shipper box and the manufacturer's closing joint must remain intact. Minor damage is allowed on the internal/ intermediate cartons.
  - No damage is allowed on the pouches.
- Post transportation test pouch sampling:
  - All pouches were inspected visually. Damaged pouches were recorded.
  - Pouches with folds/creases found on the porous top web were subjected to the microbial barrier test (samples selected by the laboratory).

Microbial barrier testing results

Three of the six pouch materials—60g Paper / 11g Grid Lacquer; 80g Reinforced Paper / 3g Allover Coating; and 85g Reinforced Paper / 11.5g Allover Coating—showed the largest decrease in microbial barrier performance, especially post Gamma and transportation testing. For those three medical-grade paper types, creases and punctures were the main cause for the deterioration in microbial barrier. Some very high % pMax values (up to 100% for single values) gave an indication of punctures, meaning integrity failures, which have been confirmed via additional visual examination (see Figures 9-11).

The same three medical-grade paper types (60g Paper / 11g Grid Lacquer; 80g Reinforced Paper / 3g Allover Coating; and 85g Reinforced Paper / 11.5g Allover Coating), had the poorest overall microbial barrier performance, post all environments, including pre-sterilization.

Two of the six pouch materials—Uncoated Tyvek® 1073B and Uncoated Tyvek® 2FS™—have shown the best microbial barrier performance overall, compared to the four medical-grade paper types.

As shown in Figure 6, several pouch material types had some decrease in microbial barrier performance post-sterilization (EO and Gamma) and environmental conditioning and transportation testing compared to pre-sterilization. However, material punctures had the highest negative impact.

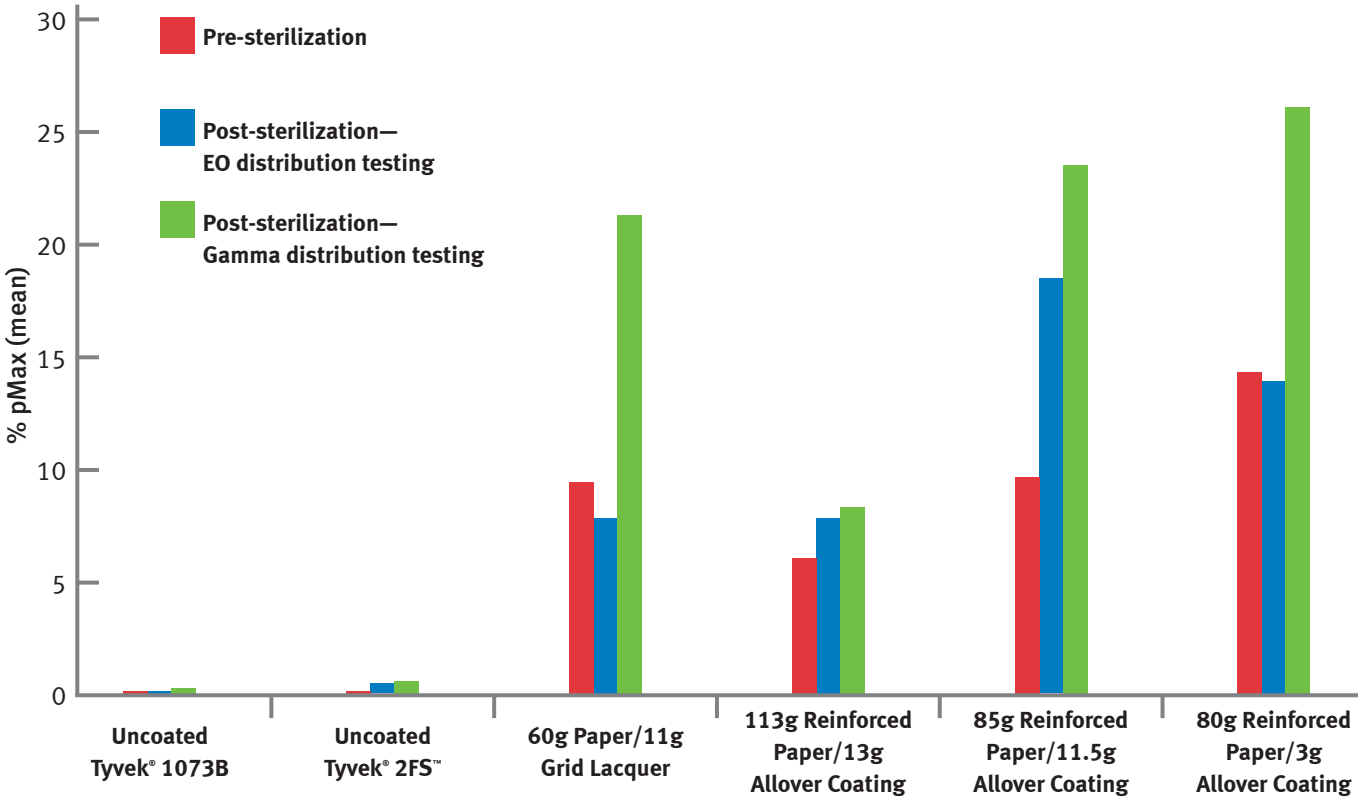

Figure 6. Microbial barrier performance post all environments, including pre-sterilization, as tested according to ASTM F2638. Results are shown in percent maximum penetration (% pMax). The lower the % pMax, the better the performance.

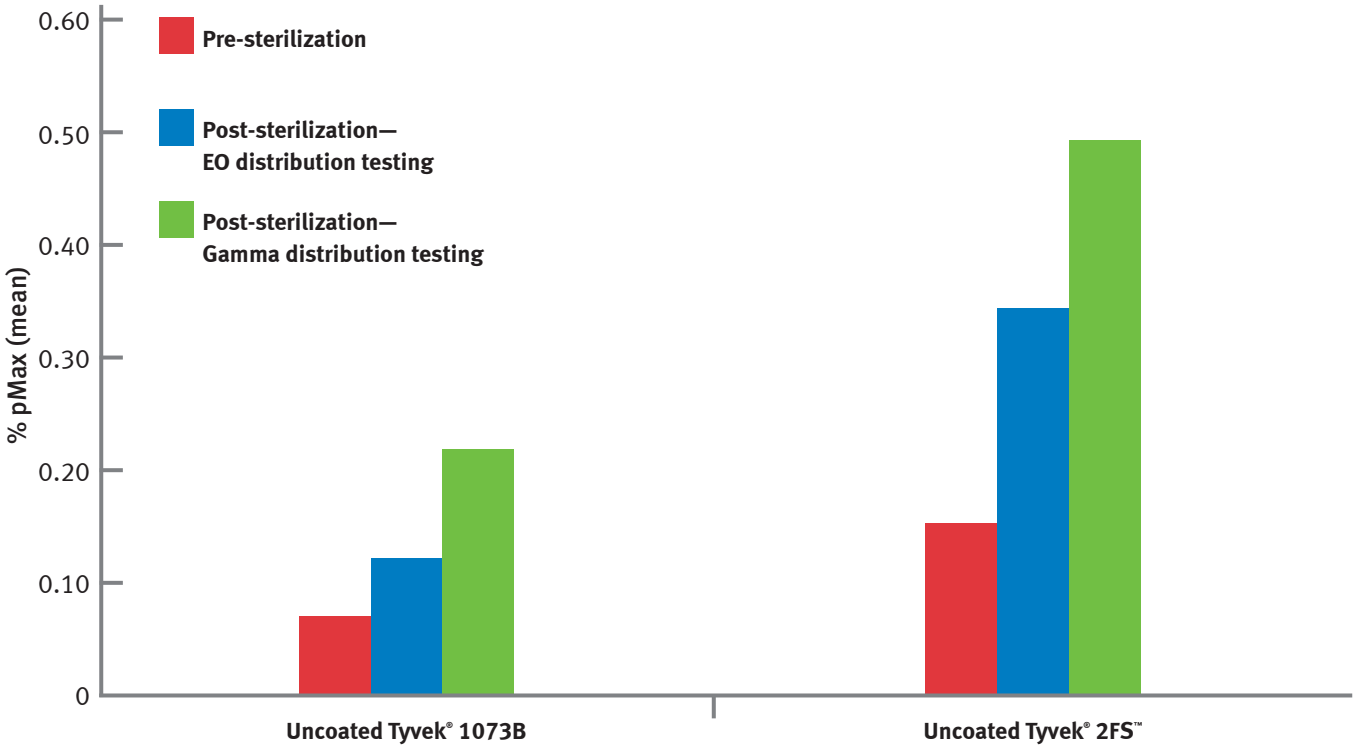

Figure 7. Microbial barrier performance post all environments, including pre-sterilization, as tested according to ASTM F2638, for Tyvek® styles only. The scale of the Y axis has been magnified (compared to Figure 6) to show detail. It is important to note that Tyvek® has a pMax of less than 0.5% in all conditions tested; whereas, the pMax for the four medical-grade papers in this study range from approximately 6% to approximately 26% (as shown in Figure 6).

To evaluate the potential change in % pMax for the various environments, the difference between pre-sterilization versus post-sterilization (EO and Gamma) and environmental conditioning and transportation testing was calculated using the formula shown here:

MB (Microbial Barrier) difference (% pMax) = MB post-sterilization transport (% pMax) – MB pre-sterilization (% pMax)

The mean is then calculated
(out of 10 measurements MB difference per condition).

Result interpretation:

- → If MB difference mean value is >0 (positive) = pMax has increased post-sterilization (EO or Gamma) and environmental conditioning and transportation testing, which means the microbial barrier performance got worse
- → If MB difference mean value is <0 (negative) = pMax has decreased post-sterilization (EO or Gamma) and environmental conditioning and transportation testing, which means the microbial barrier performance got better
- → If MB difference mean value is =/≈ 0 (equal or next to zero) = no/only slight change in pMax or barrier performance post-sterilization (EO or Gamma) and environmental conditioning and transportation testing

The analysis has been made using Minitab® statistical software. The results are displayed in boxplot graphs (see Figure 8).

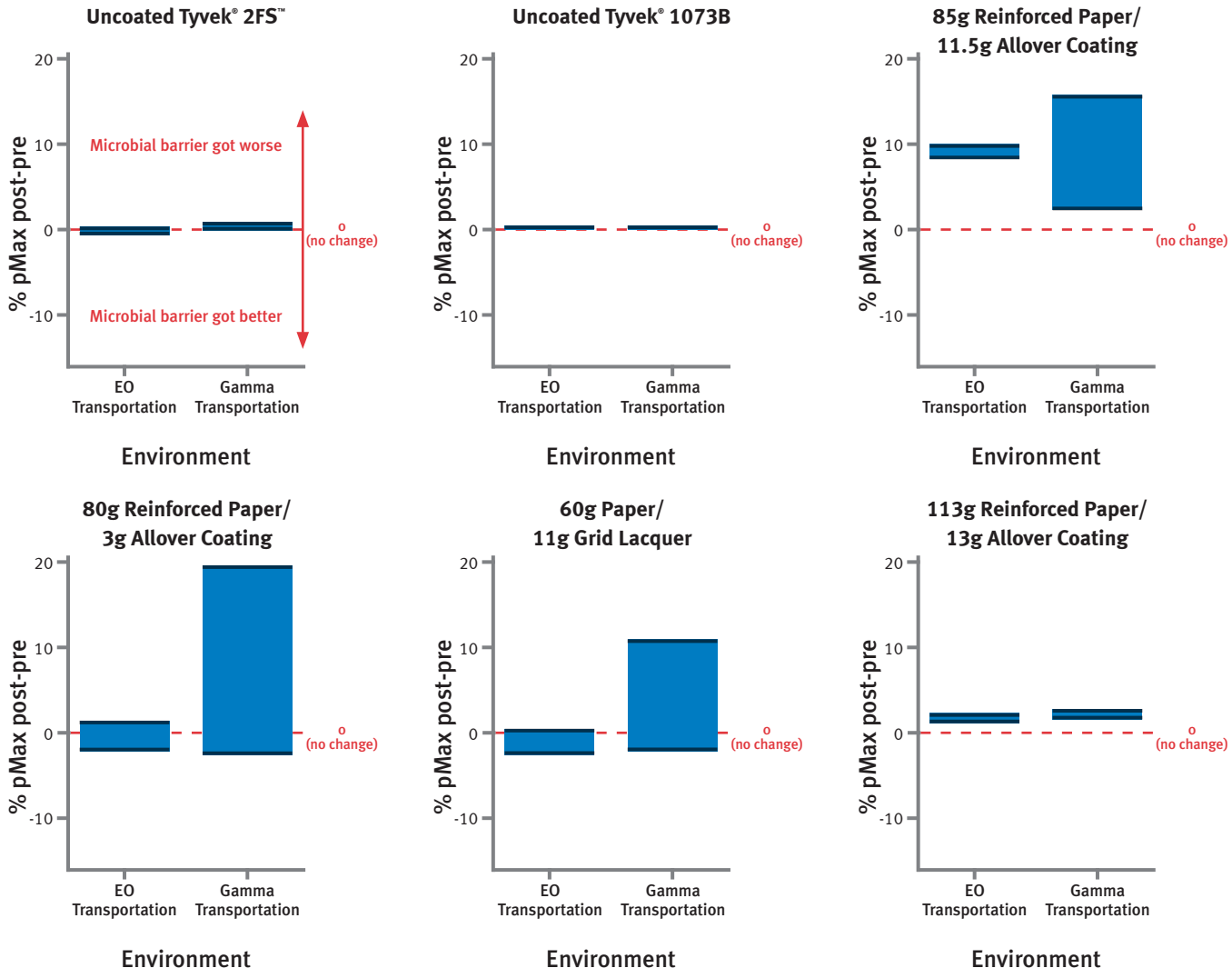

Figure 8. Boxplot for microbial barrier difference in % pMax post-sterilization (EO and Gamma) and environmental conditioning and transportation testing.

The boxplot graphs shown in Figure 8 confirm that the microbial barrier performance of some of the materials decreased to some extent post-sterilization (EO and Gamma) and environmental conditioning and transportation testing compared to pre-sterilization.

Please note that some of the single values may not be visible (mostly outliers related to material punctures) because the scale has been adapted to simplify the reading.

The high maximum penetration results reported for three of the medical-grade paper types post-sterilization Gamma and environmental conditioning and transportation testing indicate that Gamma radiation has some negative impact on the microbial barrier performance of some medical-grade papers. Furthermore, the data is showing a higher variability of results post Gamma and environmental conditioning and transportation testing on the same three medical-grade papers, mainly linked to the confirmed punctures in the material (see Figures 9-11).

In addition, 113g Reinforced Paper / 13g Allover Coating and Tyvek® show some slight decrease in microbial barrier, especially post Gamma—with the decrease for Tyvek® being below one % point (the overall maximum penetration itself is already that low compared to the other tested materials), as shown in Figure 7. The reason for this slight decrease in microbial barrier may be that radiation has some influence on the electrostatic charge of materials.[14]

It is also important to note that, in general, the acceptance limit for microbial barrier performance pre-sterilization, as well as for changes in microbial barrier performance post different environments, varies depending on the specific application requirements and defined risk level associated with the packaged device. ASTM F2638 is a very useful test to rank and qualify porous materials and to assess the risk of potential changes to the microbial barrier performance linked to specific occurrences.

Coating usually has a positive impact on barrier performance but may reduce porosity, which is a critical factor for gas sterilization. In this study, data has shown that Tyvek®, which consists of continuous high-density polyethylene (HDPE) filaments, has the best microbial barrier performance even though the samples had no coating applied, unlike the medical-grade papers tested within the study, which had either an allover or grid lacquer coating applied. Refer to Table I.

Figure 9. This pouch made with 60g Paper / 11g Grid Lacquer had a confirmed puncture.

Figure 10. This pouch made with 85g Reinforced Paper / 11.5g Allover Coating had a confirmed puncture.

Figure 11. This pouch made with 80g Reinforced Paper / 3g Allover Coating had a confirmed puncture.

Although punctures, such as those shown in Figures 9-11, can be easily seen during visual inspection, it is often very difficult to determine if there are micro punctures present. Microscopic photographs demonstrate this point (see Figures 12 and 13). Micro punctures, which are linked to creases or other mechanical impact, can potentially result in loss of package integrity. Microbial barrier testing is a useful way to check any uncertainties.

Figure 12. Microscopic photograph (175x magnification) of creases and potential puncture in pouch made with 60g Paper / 11g Grid Lacquer. The measured % pMax of this sample was 12.22 %.

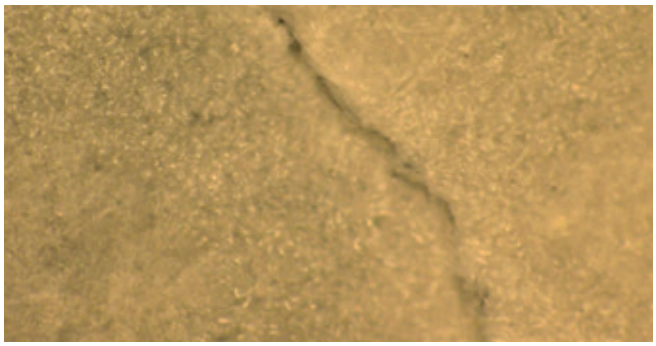

Figure 13. Microscopic photograph (175x magnification) of potential puncture in pouch made with 60g Paper / 11g Grid Lacquer. The measured % pMax of this sample was 100 %.

Conclusions

The data generated for this study showed that on several porous packaging materials, barrier performance decreased to some extent post-sterilization (EO and Gamma), environmental conditioning and transportation testing compared to pre-sterilization.

Three different medical-grade paper types, out of the six pouch materials tested, showed the largest microbial barrier performance decrease, especially post Gamma and transportation testing. Of those three medical-grade paper types, creases and punctures were the main cause of the deterioration in microbial barrier. Gamma radiation has a negative impact on the microbial barrier performance of some paper materials.

The same three medical-grade paper types also had the poorest overall microbial barrier performance post all environments, including pre-sterilization.

Uncoated Tyvek® 1073B and Uncoated Tyvek® 2FS™ have shown the best microbial barrier performance overall compared to the four medical-grade paper types in this study.

Microbial barrier testing is essential to rank and qualify porous materials and to assess the risk of potential changes to the microbial barrier performance (e.g., to detect micro punctures) linked to specific occurrences, such as transportation testing. This study could be extended to microbial barrier testing post stability testing, as aging may have an influence on the microbial barrier properties of materials as well.

Because common package integrity tests are limited in their leak detection sensitivity, microbial barrier testing will provide additional and more detailed input to assist in judging overall package integrity, while responding to the requirements as outlined in ISO 11607.

The study also shows that an adequate testing strategy is essential to ensure a safe and reliable sterile barrier system (SBS), thereby supporting patient safety.

Test Methods

Standard / Reference	Description
ASTM D4169-09	Standard Practice for Performance Testing of Shipping Containers and Systems
ASTM D4332-01	Standard Practice for Conditioning Containers, Packages, or Packaging Components for Testing
ASTM D4728-06	Standard Test Method for Random Vibration Testing of Shipping Containers Method A
ASTM D5276-98	Standard Test Method for Drop Test of Loaded Containers by Free Fall
ASTM D642-00	Standard Test Method for Determining Compressive Resistance of Shipping Containers, Components, and Unit Loads
ASTM D999-08	Standard Methods for Vibration Testing of Shipping Containers Method A1
ASTM F1886-09	Standard Test Method for Determining Integrity of Seals for Medical Packaging by Visual Inspection
ASTM F1929-98	Standard Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration
ASTM F1980-07	Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices
ASTM F2096-04	Standard Test Method for Detecting Gross Leaks in Medical Packaging by Internal Pressurization (Bubble Test)
ASTM F2638-12	Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier
ASTM F88/F88M-09	Standard Test Method for Seal Strength of Flexible Barrier Materials
ISO 2233:2000	Packaging -- Complete, filled transport packages and unit loads -- Conditioning for testing
ISTA 2A:2011	Simulation test for individual packaged-products less than 150 lbs

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The information provided herein corresponds to our knowledge on the subject at the date of its publication. This information may be subject to revision as new knowledge and experience becomes available. The data provided fall within the normal range of product properties and relate only to the specific material designated; these data may not be valid for such material used in combination with any other materials or additives or in any process, unless expressly indicated otherwise. The data provided should not be used to establish specification limits or used alone as the basis of design; they are not intended to substitute for any testing you may need to conduct to determine for yourself the suitability of a specific material for your particular purposes. Since DuPont cannot anticipate all variations in actual end-use conditions DuPont makes no warranties and assumes no liability in connection with any use of this information. Nothing in this publication is to be considered as a license to operate under or a recommendation to infringe any patent rights.

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