8-2009

THE EFFECT OF RELATIVE HUMIDITY ON THE MICROBIAL BARRIER PROPERTIES OF POROUS PACKAGING MATERIALS USED IN THE MEDICAL DEVICE INDUSTRY

Jennifer Blocher
Clemson University, jen.blocher@gmail.com

THE EFFECT OF RELATIVE HUMIDITY ON THE MICROBIAL BARRIER PROPERTIES OF POROUS PACKAGING MATERIALS USED IN THE MEDICAL DEVICE INDUSTRY

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Packaging Science

---

by
Jennifer Blocher
August 2009

Accepted by:
Dr. Duncan O. Darby, Committee Chair
Dr. Kay Cooksey
Dr. Laura Bix

ABSTRACT

Porous packaging materials, such as medical-grade paper and Tyvek®, are used often by the medical device industry for packaging sterile devices when the products are sterilized post packaging. Their use can be attributed to the fact that multiple sterilization methods require the ability for vapors to enter and/or exit the package efficiently, while simultaneously reducing the amount of microbes entering the package. Much research as been done to study the effects of multiple material and environmental factors, such as material structure and dispersion concentration, on the microbial barrier properties of these materials, however no research had been conducted to examine the impact of relative humidity. This research was aimed at identifying the effect relative humidity levels can have on the microbial barriers of four porous packaging materials: coated and uncoated Tyvek® 1073B, dot coated Ovantex®, and coated medical-grade paper. Research was conducted with slight adjustments to the ASTM F2638-07 test standard method for using aerosol filtration for measuring the performance of porous packaging materials as a surrogate microbial barrier. The adjustments included preconditioning samples at 15%, 50%, and 90% relative humidity levels and switching samples after each tested flow rate. Results from testing show that the microbial barrier properties of medical-grade paper were significantly impacted by fluctuations in relative humidity. Microbial barrier properties of the medical-grade paper and Ovantex® were also significantly impacted by the dispersion flow rate through the material sample, while uncoated Tyvek® 1073B was found to only be slightly impacted.

Interestingly, when analyzing the coated and uncoated Tyvek®, a data analysis suggests that the addition of the heat seal coating may significantly decrease the impact flow rate has on microbial penetration.

ACKNOWLEDGEMENTS

I would like to thank my core advisor, Dr. Duncan Darby, and other members of my thesis committee, Dr. Kay Cooksey and Dr. Laura Bix, for their help, guidance, and support throughout this project.

A thank you to Dr. Patrick Gerrard for his help and expertise with experiment design and analysis.

I would like to thank Sueanne Belmonte (Perfecseal) for donating much of the material required by my project.

Katey Zinn (Mangar) for providing materials and industry support.

Curt Larsen and John Spitzley (Spartan Design Group) and Tim Early (Packaging MD) for their industry support.

Paul Herman (DuPont) for allowing me the use of the testing equipment and facilities.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In 2002, a “Surgical and Medical Equipment” marketing survey valued the medical device industry at $140 billion and rapidly growing (“Surgical and Medical Equipment,” 2009). Manufacturers produce a wide variety of products ranging from diagnostic equipment and x-ray machines to therapeutic devices and cardiac catheters.

The sterility of most medical device products is of paramount importance. Companies are expected to design a packaging system that will protect their product from manufacture, through distribution channels, to point of use. Terminally sterile devices, products sterilized while inside a sealed package, also require consideration of the sterilization method and in some cases must be designed to facilitate the sterilization process.

Several techniques can be used to sterilize medical products. Each sterilization method adds different constraints, requirements, and stresses to the packaging system. Because some sterilization methods such as Ethylene Oxide (EtO), Vaporized Hydrogen Peroxide (VHP), and steam autoclave use gases or vapors to achieve sterility, porous materials, such as medical grade papers and Tyvek®, have become the prominent packaging materials in the medical device industry. Porous materials allow gases and vapors to enter and exit the package while simultaneously inhibiting microbes from entering the package. There are several theories as to how the reduction of microbial ingress is achieved within porous materials. Many suggest the causes for reduction are attributed to materials acting as a depth filter or creating a tortuous path that traps microbes

as the air weaves through the material (DuPont,19) (Permeability, 65). With porous materials playing such a large and important role in medical device packaging, it is important that the industry fully understands the microbial barrier properties of these materials and how they are affected throughout the distribution cycle.

Currently, most data on the microbial penetration levels of these materials have been determined using ASTM F1608-00: Standard Test for Microbial Ranking of Porous Packaging Materials (Exposure Chamber Method). In this test method, Section 8- Sample Preparation states that the test can be performed on materials “before or after they are subjected to other conditions such as heat or cold, relative humidity, different sterilization processes, real time, or accelerated aging.” Although preconditioning is permissible following the current ASTM methods, there is limited information available that discusses the effects of relative humidity levels on the microbial barrier properties of materials typically employed to package terminally sterile medical devices. Since significant changes to relative humidity levels during the packaging distribution cycle occur, it is important to research how relative humidity levels affect the microbial penetration levels and potentially the sterility of medical device products.

This research analyzes the microbial barrier performance of porous packaging materials conditioned under controlled atmospheric conditions. It was designed to assist in forming a baseline understanding of how relative humidity affects microbial barrier properties of the tested porous materials.

To ensure that the optimum penetration flow rate for each material was tested, research testing was conducted using ASTM F2638-07: Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier as a guideline with slight variations to sample preparation. Unlike the ASTM F1608-00 standard, the ASTM F2638-07 standard varies the challenge flow rates in order to test the sample at the optimum flow rate for microbial penetration through the sample. Four different medical grade porous packaging materials were subjected to testing: Tyvek® 1073B, coated Tyvek® 1073B, dot coated Ovantex®, and coated 55 pound medical-grade paper. Materials were preconditioned and tested with slight modifications to the ASTM F2638-07 test method. All data was then studied to determine if there was any correlation between the relative humidity levels, flow rates, and the microbial barrier properties for each of the four materials tested.

REVIEW OF LITERATURE

Medical Devices

When researching medical device packaging, it is important to first understand what constitutes a medical device. Defining a medical device can be complicated when one considers that different countries define devices differently. For this reason, the International Standards Organization (ISO) has defined a medical device as

"any instrument, apparatus, implement, machine, appliance, implant, in vitro reagent or calibrator, software, material or other related article, intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the specific purpose(s) of

- Diagnosis, prevention, monitoring, treatment or alleviation of disease
- Diagnosis, monitoring, treatment, alleviation of or compensation for an injury
- Investigation, replacement, modification or support of the anatomy or of a physiological process
- Supporting or sustaining life
- Control of conception
- Disinfection of medical devices
- Providing information for medical purposes by means of in vitro examination of specimens derived from the human body

And which does not achieve its primary intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means (ISO 11607-1, 2)."

Under this definition, medical devices encompass a large range of products. "Complicated capital equipment, such as MRI tunnels and x-ray machines are medical devices, but so are simple, commodity-like items

such as tongue depressors and syringes. Some are meant for mass markets, others are niche items. Some are packaged individually; others are packaged in boxes of 100s or 1,000s. Some are reprocessed, others disposable, and some are used for a lifetime (Bix, 1)." Risks associated with device misuse and failures are equally varied, ranging from inconvenience to patient death.

Such a broad range of products and risks make it necessary for the Food and Drug Administration (FDA) to segregate devices into varied categories, or product classes. There are three classifications that are recognized by the FDA. Class I products are where "general controls are sufficient to provide reasonable assurance of the safety and effectiveness of the device" and are not life-supporting or life-sustaining (21CFR860.3). These devices are for a "use which is of substantial importance in preventing impairment of human health, and which does not present a potential unreasonable risk of illness or injury. (21CFR860.3)" Class II products are devices where "general controls alone are insufficient to provide reasonable assurance of its safety and effectiveness" and are "to be for use in supporting or sustaining human life (21CFR860.3)." Class III products require premarket approval as they have significantly higher risk rates than Class I and II devices. These devices are life-supporting or life-sustaining, or for a use which is of substantial importance in preventing impairment of human health, or if the device presents a potential unreasonable risk of illness or injury (21CFR860.3)." Manufacturers must classify all their products according to the

FDA product classes and follow the design testing and validation procedures specific to the product class.

Medical Device Industry

The medical device industry across the globe was valued as a $140 billion business in 2002 with an anticipated growth of 5 percent through 2010 (“Surgical and Medical Equipment,” 7). Today, the United States remains the world’s largest consumer of medical products, at 40 percent, and the largest producer, manufacturing 50 percent of all medical devices.

The orthopedic (pertaining to the musculoskeletal system) market segment alone was valued at $20 billion worldwide in 2005. This segment has experienced rapid market growth of 13-15 percent during the mid-2000s. Growth in orthopedics is expected to continue and has been attributed to the aging population, longer life spans, and higher rates of obesity (“Surgical and Medical Equipment,” 8).

Although the US holds the position of world leader as both a manufacturer and consumer of medical equipment, Western Europe, Japan, and China are closing the gap. The European market is anticipated to grow to a value of $37.2 billion by 2010. Medical technology and manufacturing capabilities in China are growing at such a fast rate that it is expected to surpass the US in medical equipment revenues by 2039 (Surgical and Medical equipment, 16).

Major manufacturers in the medical device industry include companies such as Johnson & Johnson, Boston Scientific Corporation, Roche Diagnostics, Becton Dickinson Company (BD), and Medtronic (“Surgical and Medical

Equipment," 11-15). Each of these companies has managed to capture a leading position in a specific market within the medical device industry. Johnson & Johnson, the largest player in the industry, manages several companies that target multiple segmented markets. Johnson & Johnson companies include Depuy, which specializes in orthopedic joint reconstruction and spinal care products (Hoover's, 2009) and Ethicon, a leader in sutures and wound management. Boston Scientific produces catheters, endoscopes, and laparoscopes for the vascular and cardiovascular market segments. Roche Diagnostics was formed by a merger between the Swiss company Roche and the German company Boehringer Mannheim, and it accounts for 20 percent of the world market in medical diagnostic equipment. Although Becton Dickinson (BD) is a diverse organization servicing multiple industry segments, they are the market leaders in the needle and syringe market. Medtronic leads the world in implantable biomedical devices, specializing mainly in pacemakers and defibrillators. ("Surgical and Medical Equipment," 11-15).

Medical Packaging Types

Due to a broad range of medical products, options for medical packaging are abundant. The medical packaging industry is frequently categorized in the following ways: reusable vs. disposable and sterile vs. non-sterile. The focus of this research is on sterile, disposable devices that are terminally sterilized.

Packaging for Disposable Devices

Disposable medical devices can be sold by the manufacturer as sterile or non-sterile products and are designed for a single-use application.

Tray

Trays used in the medical packaging industry can be manufactured as rigid or flexible units that are typically comprised of a silicon coated or uncoated polyethylene terephthalate (PET) or a glycol-modified polyethylene terephthalate (PETG) base. Such trays, Figures 1 and 2, are frequently covered using a porous material, such as medical paper or Tyvek®, that enables the passage of gas sterilant, such at EtO, for terminally sterilized product. A silicon coating is sometimes used to prevent preformed trays from sticking together when stacked, or nested, for storage and distribution. Lid stock is often coated with heat seal coating to promote sealing to the trays.

Figure 1: Snap-fit rigid tray

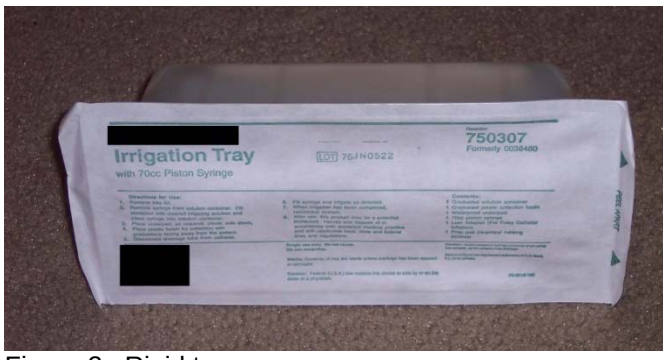


Figure 2: Rigid tray

Packaging operations using trays can be managed in one of two ways: fill and seal or form/fill/seal. Form/fill/seal operations offer manufactures inventory and space reduction, increased speeds, and more flexibility within their operations by providing the option of forming rigid or flexible trays. Fill and seal operations require the storage of preformed rigid trays, but can provide a cost-effective solution for small volume production.

Pouch

Due to their low cost, pouches are by far the most common method for packaging high-volume medical products (Sherman, 68). Many polymer structures can be used for pouches, but a dominant material combination used is a PET/LDPE (low density polyethylene) structure for one side that is then sealed to a coated or uncoated porous material, such as medical-grade paper or Tyvek®. Other popular structures contain Nylon or foil. Pouch design is strongly dependent on sterilization technique. As described for trays, manufacturers choosing pouches for their operations have the option to purchase preformed pouches for fill/seal operations or to use roll stock for form/fill/seal operations.

Two main sealing configurations are popular for pouches used in the medical industry, corner peel and chevron. Corner peel pouches offer the user a grip point on one or both corners of the pouch to begin the peel. However, the corner peel pouch (Figure 3) requires that users begin peeling at full seal width, which requires more force to initiate opening. The chevron pouch offers a smaller initial peel width, which reduces the amount of initial force required to open the pouch (Figure 4). Unlike corner peel and chevron pouches, tear pouches (Figure 6) offer easy access opening by ripping the material. Tyvek® and paper structures are not ideal tear pouch materials due to either the material strength or particulate concerns.

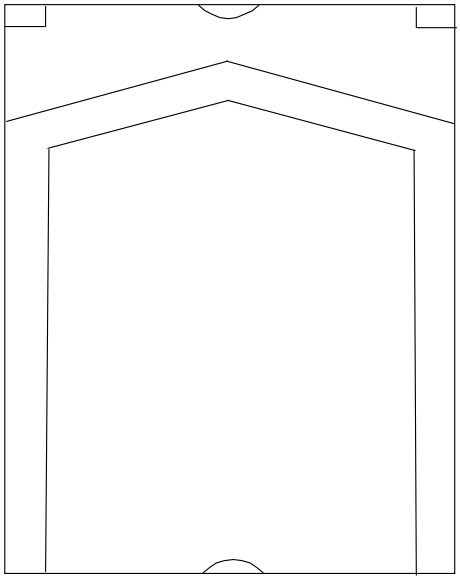


Figure 3: Chevron pouch

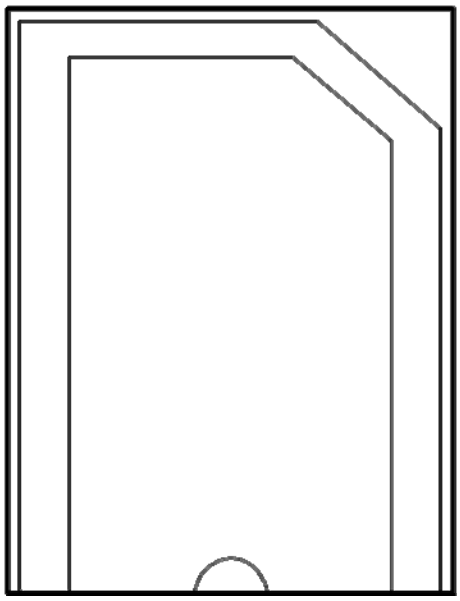


Figure 4: Corner peel pouch

Header Bag

Header bags, sometimes called breather bags, are a pouch variation that reduces the use of porous packaging materials, which in turn reduces production costs (Figure 5). With over three quarters of the pouch being made-of non-porous materials, this pouch offers porosity with increased seal and puncture strengths. These unique structures require the user to open the bag from a flap located on the interior of the pouch structure and then fold the porous material under the product to achieve aseptic presentation. Aseptic presentation is the act of the nurses opening and handling a sterile package in a manner that will not compromise the sterility of its contents.

Figure 5: Header bag

Figure 6: Tear pouch

Single vs. Double Barrier

All previously discussed methods for packaging can be used as stand alone packages or combined to form a double barrier. The term “double barrier” refers to two barriers that can be considered sterile barriers by the end user. Most end users, nurses, of medical packaging prefer double barrier packages over single barrier, because it allows them easier aseptic presentation into the sterile field (J. Neid and J. Blocher, Healthpack presentation, March 2009). Tertiary packaging, such as paperboard boxes, are not considered a second barrier by the medical packaging industry.

Reusable

Medical devices that are reusable are designed to be sterilized within the hospital system (ie. instruments) and as such, they do not require the sterility measures as the terminally sterilized products. Without the need to pre-sterilize product, reusable devices can be packaged in a multitude of ways. Many of the packaging options previously discussed are also used to package reusable

devices. However, heavier devices or devices that require more cushioning may use foam cutouts, foam-in-place, or corrugate structures to cushion and/or restrain reusable devices during distribution.

Materials

Tyvek®

Tyvek® is a unique material that offers water resistance while maintaining strength and permeability. In 1955, DuPont researcher Jim White, discovered Tyvek® by accident when he noticed a "polyethylene fluff" escaping a pipe in the research labs (DuPont, 3). Although Tyvek® looks and feels similar to paper, it is constructed from pure high-density polyethylene (HDPE) fibers and maintains a higher strength-to-weight ratio than paper. Like paper, Tyvek® is 100% recyclable given an appropriate recycling program. Recycling programs that accept the SPI #2 symbol for HDPE are capable of recycling most Tyvek® materials (DuPont, 3).

While its recyclability makes it attractive to companies striving to produce more sustainable products, it is Tyvek®'s ability to maintain strength while offering porosity and moisture resistance that make it attractive to the medical packaging industry. Porosity is a necessity for any manufacturer wishing to sterilize via Ethylene Oxide (EtO), which is the sterilization choice for approximately 50% of all disposable medical device products ("Sterilization (microbiology)," 2009).

Tyvek® is manufactured and distributed worldwide solely by E.I. DuPont de Nemours and Company, Wilmington, Delaware. Although DuPont sells

Tyvek® directly, sales and distribution of the substrate mostly occurs through converters, such as Perfecseal and Mangar, who provide the medical device industry with various forms of packaging for terminally sterilized devices. (DuPont)

There are four grades of Tyvek® that are considered acceptable for the medical device industry: 1073B, 2FS, 1059B, and Asuron. Each of the grades offers variation in microbial barrier, porosity, thickness, and many other properties. Asuron is the only grade that is not 100% HDPE. It contains titanium dioxide (TiO_2) to improve appearance of structure uniformity. (DuPont)

Manufacturing Process

DuPont has a patented process, known as flash-spinning, for the manufacture of all Tyvek® grades, Figure 7. The process begins with spinneret extrusion technology. Unlike the textile spinneret extrusion processing this spinning process involves a continuous strand of material which is used to create a non-woven structure of synthetic fibers.

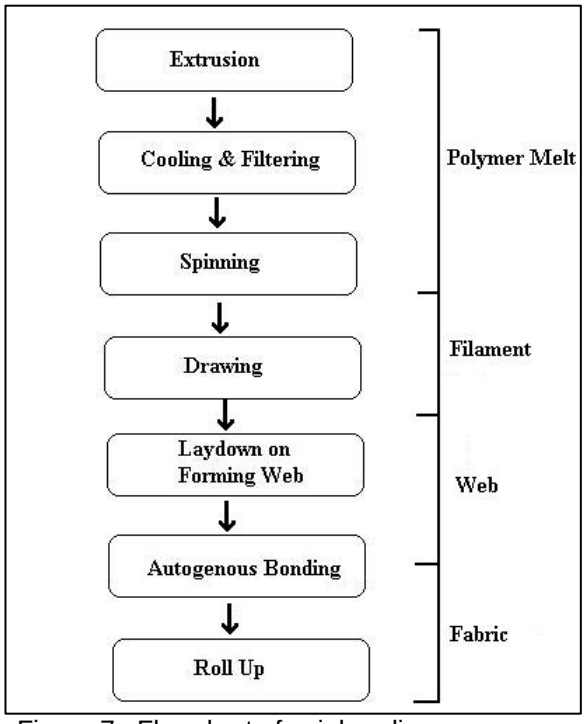


Figure 7: Flowchart of spinbonding process

A non-woven structure is defined by Wikipedia as a “term used in the textile manufacturing industry to denote fabrics, such as felt, which are neither woven nor knitted.” Initially, the synthetic material is melted, filtered for impurities, and extruded through tiny holes in the spinneret as shown in Figures 8-9 (FiberSource, pars. 1-4).

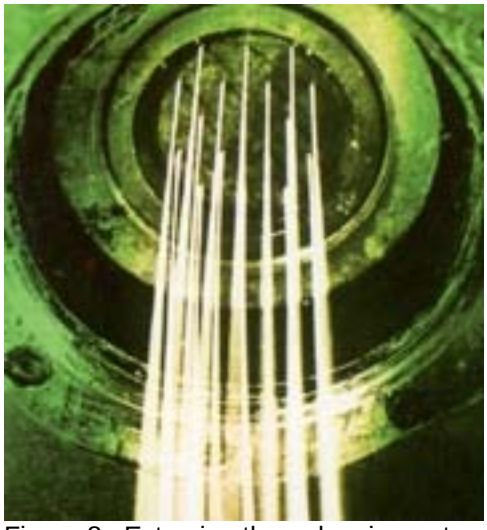


Figure 8: Extrusion through spinneret
Photo used with permission from AKZO Nobel

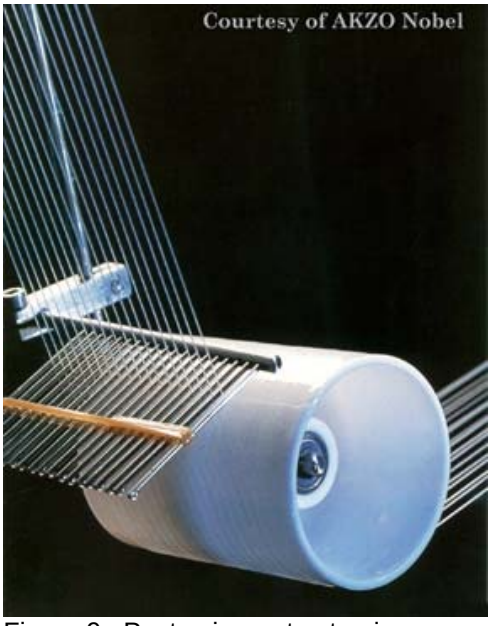


Figure 9: Post spinneret extrusion
Photo used with permission from AKZO Nobel

DuPont's proprietary manufacturing process involves extrusion with the use of a solvent that immediately evaporates from the extruded polyethylene. The solidified substance is then laid onto a moving belt with the aid of a computerized system that optimizes "random" layering of the web, thus creating a torturous path (Figure 10) through the material. The web is then bonded together using heat and pressure.

Figure 10: Tyvek® (200x magnification)

Tyvek® grades are varied by using the same manufacturing process and varying the belt speeds and the heat and pressure variables. Unlike other forms of Tyvek®, medical-grades are not corona treated or anti-static treated because these treatments are suspected to cause potential concerns with the material properties (DuPont, 3).

Ovantex®

Launched in April 2006, Ovantex® is a newer medical-grade packaging material converted and marketed by Oliver® Medical. Designed to be both porous and sterilizable, Ovantex® is marketed as a low cost alternative to medical-grade paper and Tyvek® for medical pouching and lidding applications. Oliver® recommends the potential use of Ovantex® for such products as sutures, syringes, irrigation sets, wound dressings, orthopedics, cannulas, and device accessories (Oliver®, www.oliver-tolas.com).

Manufacturing Process

Although the exact method to manufacture Ovantex® remains undisclosed, Oliver® states that Ovantex® is composed of a cellulose and synthetic fiber web (Oliver®, Ovantex® Facts). Such a process would result in a medical-grade paper laced with an unknown synthetic fiber in a non-woven structure.

Medical-Grade Paper

Little to no information is available to distinguish medical-grade paper from other paper grades, however there is speculation that the determination is based

on fiber length (to reduce particulate concerns), puncture and tear resistance, and porosity.

Paper is manufactured through a set of basic manufacturing processes. The process begins with pulping, which breaks down the wood into its fibers. The fibers are then suspended in water and refined. Additives, such as fillers, are then added. The slurry is then placed on a mat, drained of excess water, and dried thoroughly. (Bowyer)

Sterilization Methods

Ethylene Oxide (EtO)

Ethylene oxide is a colorless, odorless, highly flammable, and explosive gas that the medical device industry and hospital systems use to achieve product sterilization at reasonably low temperatures. Sterilization at low temperatures makes EtO sterilization very attractive for heat sensitive products. Five main variables are used to achieve safe and effective EtO sterilization: Gas mixture, gas concentration, temperature, humidity, and exposure time (Dyro, 534).

To reduce the explosive properties of EtO, it is often combined with other gasses, such as Nitrogen (N_2), Carbon Dioxide (CO_2), or Hydrochlorofluorocarbon (HCFC) (Sherman, 160). For CO_2 to render EtO inert, 90% of the gas mixture must be CO_2 which greatly diminishes the EtO potency. HCFC mixtures allow for safe sterilization while maintaining a higher concentration of EtO, but is expensive and hazardous to the environment. For these reasons, most sterilizers use 100% EtO that is later neutralized in the sterilization chamber with N_2. This method requires a significant investment in

capital to improve equipment safety measures, but also allows manufacturers to reduce their cycle times.

In 1949, Kaye and Phillips established that, as relative humidity (RH) levels increase, sterilization through EtO is achieved much faster (Sherman, 158). For this reason, sterilizers often precondition products to an RH of 70% and conduct sterilization at the same RH level.

Biological Indicators (BI's) are used to ensure the lethality of the sterilization cycle and to eliminate the need for actual sterility testing on product from daily production runs. A typical BI is made from paper that has been impregnated with a known population of bacterial spores of a certified resistance value (Sherman, 144). BI's are placed throughout the sterilization loads, especially in locations that may be difficult for vapors to penetrate.

The low temperature processing requirements to accomplish sterilization is the main advantage for EtO sterilization. The main disadvantage is the toxicity of EtO. EtO is considered by the Occupational Health and Safety Administration (OSHA) to be a "potential human carcinogen (Sherman, 170)." If not properly aerated, EtO can cause serious chemical burns, to workers, healthcare providers and patients, when it contacts skin for even a short duration. Aeration refers to allowing the product sufficient time for any hazardous residues associated with the sterilization cycle to escape the package naturally through the porous material structure. The EtO aeration cycle is dependent on many factors such as dose mixture, exposure time, product make-up, and the product's intended usage. The ANSI/AAMI/ISO 10993-7 (Standard for Biological Evaluation of

Medical Devices, Part 7: Ethylene Oxide Sterilization Residuals) outlines the acceptable residual levels and the necessary aeration times required. Aeration cycles can range from two hours to seven days, which has led many hospitals and device manufacturers to move towards alternative sterilization methods.

Gamma Radiation

Gamma radiation can be achieved through radioactive isotopes (Van de Graaf and microwave linear generator) and radioisotopes (Cobalt-60 or Cesium-137) (Sherman, 176). Both types of equipment achieve sterilization by creating free radicals that react with the nucleic acids of the microorganisms that lead to their destruction (Sherman, 173). Accounting for about 50% of the sterilization market, gamma radiation is quickly replacing Ethylene Oxide as the primary sterilization method in the medical device industry (Dyro, 534).

Unlike EtO and other gas sterilization methods, gamma radiation does not require the use of biological indicators (BIs) but instead uses dosimetry to ensure products receive the appropriate dosage of radiation. Dosimetry is the activity of using a dosimeter to measure the amount of absorbed radiation. The dosage is typically measured in gray (Gy). According to AMMI/ANSI/ISO 11137 – Sterilization of Health Care Products, 25 kGy is typically sufficient for small-volume production (Sherman, 175).

Benefits of gamma radiation include: rapid processing, system flexibility, no temperature concerns, increased throughput, and immediate availability of the product due to lack of aeration quarantine. Gamma radiation also has the added benefit of allowing manufactures more options in material selection as porous

materials are no longer a requirement. However, alternative means for sterilization are not problem-free, gamma radiation can embrittle and yellow many plastics. As a result, it is critical that all plastics are tested using the maximum possible dosage for the recommended lifetime of the product.

Steam Sterilization

For heat tolerant medical device products, steam sterilization is the most economical option for product sterilization and does not require the need for storage or handling of any dangerous compounds (Dyro, 533). Steam sterilization is frequently used in hospitals to sterilize equipment for surgery. The most common steam sterilization method is steam under pressure. For this method, an autoclave is used to remove air from the exposure chamber and then to subject a device to steam under extreme temperature and pressure variables. Steam created in the autoclave chamber transfers its thermal energy to the microorganisms, which results in their inoculation (Dyro, 533). This method remains the most economical sterilization option because it requires only water and roughly 20 minutes to complete the steam cycle. Disadvantages to this method are the requirement of BIs and the extreme temperature and pressure conditions, around 250 degrees F at 106 kPa pressure, required.

Vaporized Hydrogen Peroxide (VHP)

Vaporized hydrogen peroxide is used to sterilize surfaces by the production of a hydrogen peroxide gas cloud or low temperature plasma that surrounds the products in sterilization. The process begins by placing the sterilizable packaged product into the sterilization chamber. A vacuum is then

used to remove all air. The outer chamber is then injected with a 58% hydrogen peroxide water mixture (Dyro, 535). This mixture is vaporized and dispersed throughout the sterilization chamber. Once the mixture has been fully dispersed in the chamber, plasma is then generated through an electromagnetic field initiated through the use of a radio frequency (RF) generator.

Advantages to the VHP sterilization system include: short cycle time at approximately one hour, low temperature, eco-friendly with the only by-product being oxygen and water, less hazard to metal products than steam sterilization, and easy installation in various locations due to the standard 208-volt requirements. Disadvantages are that HP requires the use of BIs and cannot be used with several materials, (ie. nylon, cellulose, polycarbonate, etc) because the materials can become brittle or experience absorption issues (Dyro, 535). VHP is a surface sterilization technique; device design is of great importance, as the product must not have surfaces where vapor cannot penetrate.

Package Integrity

ISO 11607 Parts 1 and 2 serve as the primary guidance for determining the requirements of medical device packaging. As of 2006, this standard was updated to include Annex B, which includes recommended standard test methods that can be used to comply with the requirements of ISO 11607.

Microbial Penetration

Since porosity is a requirement for some sterilization methods, microbial penetration through the given porous material must also be studied. ASTM F1608-00: Standard Test Method for Microbial Ranking of Porous Packaging

Materials is currently the industry's accepted method, also found in ISO 11607 Annex B, for determining microbial barrier properties of porous materials. However, as of 2007, a new alternative standard was introduced, ASTM F2638-07: Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier. Both methods offer different capabilities, advantages, and disadvantages.

F1608

The F1608: Standard Test Method for 'Microbial Ranking of Porous Packaging Materials', also called the Exposure Chamber or Forced Flow Method, tests the penetration of *Bacillus subtilis* var. *niger* spores through a chamber containing five 47-50-mm diameter samples. The five samples and one control sample are placed in each of the six ports in the exposure chamber, Figure 11.

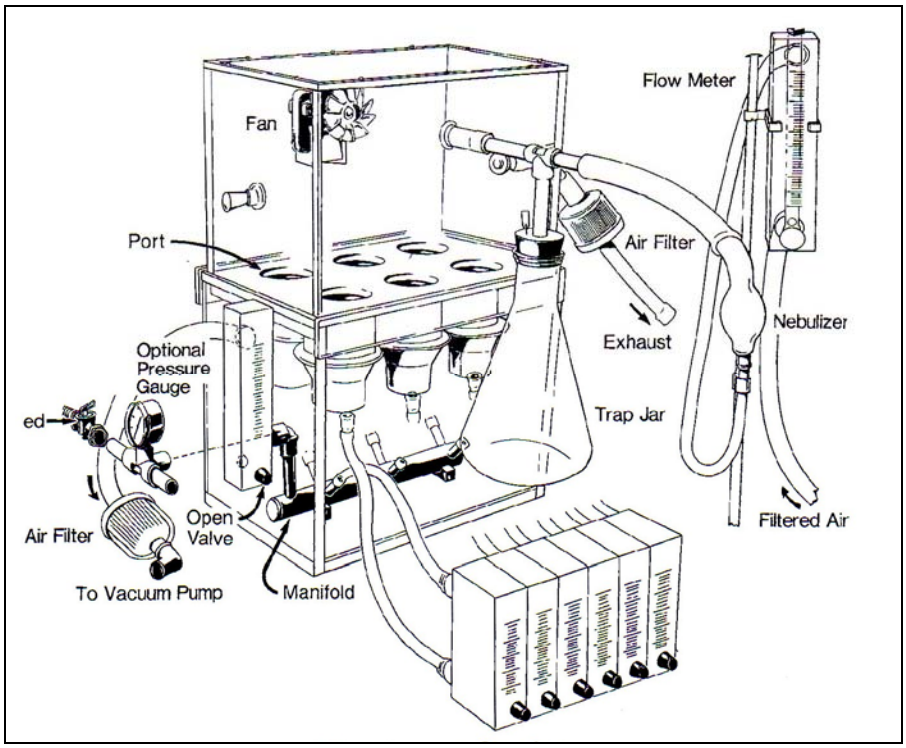


Figure 11: Exposure chamber for F1608 test standard (ASTM F1608-00)

The number of bacterial spores the samples are subjected to during testing is considered the “challenge,” while the number of the challenge spores that penetrate the sample is called the “filtrate.” The concentration of the challenge is often measured as the number of colony forming units, or CFU. In this method, a challenge of $1x10^6$ CFU/sample/port, are forced through the porous material sample at a flow rate of 2.8 L/min for an exposure time of 15 minutes (F1608, 3-4). Samples are then incubated for a minimum of 24 hours at 30-35ºC to allow appropriate time for bacterial colonies to grow. A bacterial colony is a group of bacteria that is assumed to be derived from one bacterial spore. Following the incubation period, the number of colonies are counted, recorded, and used to calculate the log-reduction value (LRV). The LRV expresses the ability of a material to reduce penetration of the bacterial spores through the substrate and is calculated using the formula:

$$LRV = \log_{10} N_0 - \log_{10} N_1 \qquad \text{(Equation 1)}$$

Where:
N_0= avg. bacterial challenge determined from the challenge control filter, CFU
N_1= avg. number of bacteria passing through Test Sample 1, CFU. If $N_1<1$, then LRV is expressed as $>\log_{10}N_0$.
(F1608-00, section 12)

The LRV values are then used to rank the microbial penetration properties of the porous materials. Higher LRV values represent a higher microbial retention by the material. Table 1, demonstrates a material’s ability to reduce the number of bacterial spores that could enter a sterilized packaging, thus compromising the sterility of its contents.

Table 1: Log reduction value correlation to percent spore retention (ASTM F1608, Section 12.8)

LRV	Spores Retained, %
1.0	90
2.0	99
3.0	99.9
4.0	99.99

The main advantage to this test method is that the consistent flow rate allows for easy cross-material comparisons, which can aid manufacturers when choosing materials. Disadvantages are that it is a labor intensive process that is time consuming and costly. Additionally, this method has been criticized for using elevated flow rates and dispersion concentration levels that may not be representative of "real-world" applications. Additionally, the industry has indicated the use of microbes to be problematic because it requires a relatively high level of skill, and can result in variable results.

Testing with Bacterial Spores vs. Particles

In an effort to address the disadvantages of testing with ASTM F1608-00, researchers from the University of Manchester sought to determine an alternative method that was more cost effective, time efficient, and allowed for variation of flow rates for more "real-world" application.

Given that "microbial cells are highly consistent in size," the researchers sought to develop a correlation between microbial spore penetration and fixed size particle penetration through commonly used porous packaging materials ("Definition of Correlation", 12). The general idea was that particles, which can be more consistent and don't require the same degree of care in storage and handling, could be correlated to microbial penetration, simplifying the test and

decreasing the variability of results. Tallentire and Sinclair sought to find a correlation independent of fiber properties, sheet porosity, and material depth or thickness ("Definition of Correlation", 12). For this reason, they conducted their research on 28 varieties of porous materials. Twelve materials were designed by the researchers to "provide a range of structures exhibiting grade barrier performance ("Definition of Correlation", 12)," while the remaining sixteen materials were provided by industry members. The industry materials comprised of papers, coated papers, nonwovens, and coated nonwovens.

All material samples were subjected to a challenge of *Bacillus subtilis var. niger* spores at a concentration of 10^6 spores dm^{-3} and flow rates ranging from 10^{-4} to $3x10^{-1}$ dm^3 min^{-1} cm^{-2}. Material samples were changed for each flow rate challenge. The material samples were then subjected to a particulate, diethylhexylsebacate (DEHS), challenge. Particulate size ranged from 0.04-0.6 µm, and both the challenge particle number and the filtrate particle number were counted "real time" with the use of two independent particle counting systems. Any particle counting system is appropriate as long as it has the ability to distinguish between water droplets and the PSL particles. The ASTM F2638-07 test standard recommends using an optical particle counter which uses a high-intensity light source, or laser. The PSL particles will reflect the light emitted from the laser, which is detected by a photodetector, a highly-sensitive light gathering detector, and recorded into the data logging system. Data collected, using the dual particle counting system, was then used to calculate the flow rates and the

percent of penetration through the samples for both the physical and microbiological tests.

Researchers determined a correlation between physical and microbiological penetration percentages. This correlation was found by plotting the maximum particulate penetration percentage, ${}^{part}P_{max}$ (x-axis), versus the maximum spore penetration percentage, ${}^{part}P_{max}$ (y-axis), for a $4x10^{-3}$ dm^3 min^{-1} cm^{-2}. This was done for 16 commercial papers and again for the 12 designed papers to demonstrate independence from fiber properties, sheet porosity, and material thickness. The researchers concluded that "overall findings unequivocally show that the physical/microbiological barrier correlation is applicable to the diverse structures of commercial porous packaging materials. (Definition, 17)"

F2638

ASTM F2638 uses 1µm polystyrene latex (PSL) particles in place of microbes to determine the microbial penetration rate through a given material. This test standard can be performed as a single particle counter system or a dual particle counting system. In either case, a single 120mm diameter sample is exposed to a particulate challenge with a concentration of 200-8000 particles/ml. A predetermined pressure differential, or change in pressure across the sample, is held for a minimum period of 45s, once the system has stabilized, typically 2 minutes. Using a dual counter system, the particulate challenge and the particulate filtrate are measured concurrently, but for a single counter system the particle counter must be switched between the challenge and penetration counts

throughout the test. After the exposure period has elapsed, the flow rate is lowered by reducing the pressure differential by a factor of 2. Once the new system pressure has stabilized, readings consistent for 2 minutes, the test repeats testing with particulate exposure for a minimum of 45s. This process is repeated until average filtrate particle count is less than 25 particles in 60s or the pressure differential can no longer remain stable. Since industry familiarity is in analyzing the flow rate through the sample, the pressure differential across the sample is converted to flow rate, using calculations shown by Equation 3.

Penetration percentages are calculated using:

$$R(n) = [C_F(n)/C_C(n)] \times 100 \qquad \text{(Equation 2)}$$

Where:
R(n)= penetration percentages
$C_F(n)$= penetration particle count
$C_c(n)$= particulate challenge count

$$F(n) = \frac{F_t P(n)}{P_1} \qquad \text{(Equation 3)}$$

Where:

F(n)= flow rate across the sample
F_t= calibrated flow of the particle counter
P_1= pressure across the sample
P(n)= test pressure

The penetration percentage (R) and flow rates (F) are then plotted on a log-log scale, where the x axis is flow rate and the y is penetration percentage. This graph is then used to determine the apex of the best fit line. This apex corresponds to the flow rate for which maximum penetration through the sample P_{max} is achieved under these test conditions.

Microbial Barrier Comparisons

Tyvek® obtains its microbial barrier properties by creating a tortuous path that traps microbes as the air weaves through the material (DuPont, 19). This type of microbial barrier is important to allow the material to be both porous, for vapor sterilization techniques, while maintaining product sterility. Figure 12 depicts how this type of microbial barrier is achieved.

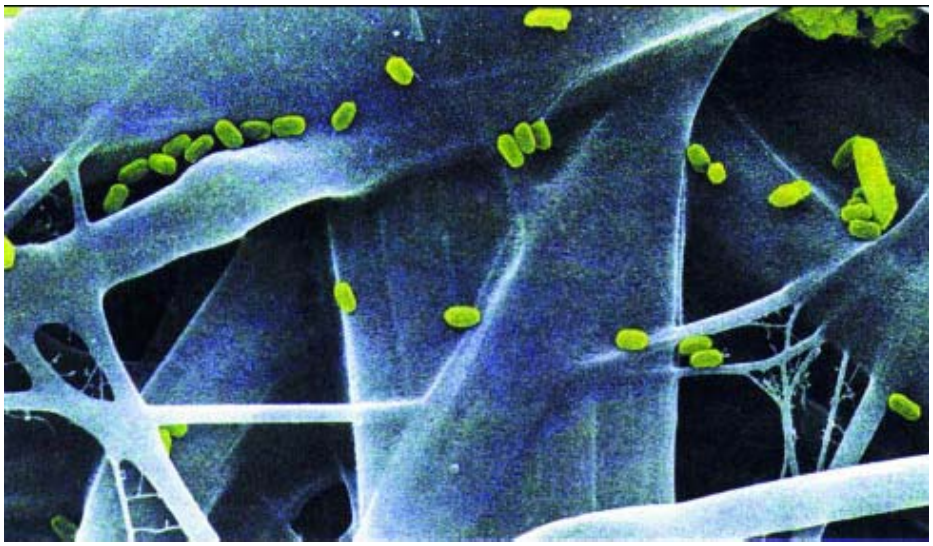


Figure 12: Microbes trapped in tortuous path of Tyvek® at 500x magnification

Both the manufacturing processes and variation in materials used to manufacturer the various porous materials are why all four materials result in different microbial barriers properties, demonstrated by Figure 13. In the past, microbial barrier data provided by the manufacturers was generated by ASTM F1608-00, a test which, as mentioned, has a constant flow rate. Microbial barrier properties can also be greatly influenced by dispersion concentration, dispersion flow rate, and material structure variations.

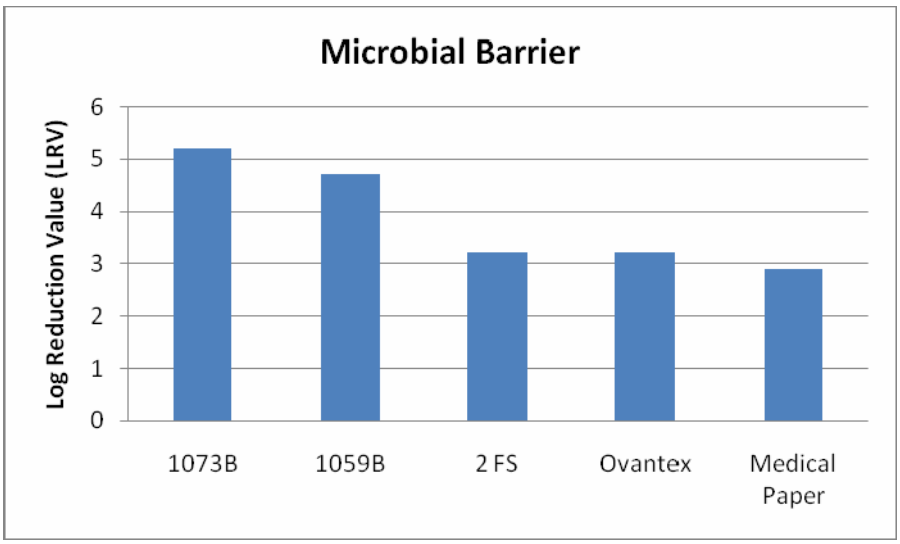


Figure 13: Microbial barrier material comparison (Exposure Chamber method) (www.dupont.com) (www.Oliver®-tolas.com)

Influence of spore dispersion concentration and dispersion flow rate on microbial barrier performance

As more research is conducted, concerns with the test methods and the test method variables come into question. When discussing microbial barrier penetration, the industry has generally accepted test method ASTM F1608-00. This method challenges porous material samples at a fixed flow rate and spore concentration level, 10^2 and 10^6 spores/L and the dispersion flow rate at 2.8 L/min for a 10 cm diameter sample.

Research by the Lewisham Hospital system (1973) was conducted to try and determine the typical concentration level of packages in multiple locations within the hospital system. The findings were that 35-65 colonies (0.19-0.36 organisms/min) were found on packages being stored in the central sterile supply area and 2-15 colonies during aseptic presentation in preparation for a surgery. The highest colony count, during this study, came from the dressing area in the

accident department (or emergency room), 120 colonies (Monty and Mayers, 18-9).

Exposing the porous packaging materials according to ASTM F1608, challenges the materials at 28,000,000 spores/min. With such a large discrepancy between the observed spore concentrations and test concentrations, the medical packaging industry is understandably concerned with the applicability of the current test standard (F1608) in the "real-world."

In 1986, Tallentire and Sinclair examined the influence of dispersion concentration in their published article ("Influence of Dispersion Concentration", 34-37). By first isolating the dynamic variables of the test, spore concentration levels and flow rate, they were then able to challenge the webs at varying spore concentrations and flow rates. Three uncoated webs were tested in the study: two commercial medical-grade papers and Tyvek® 1059B. Isolating the spore concentration and flow rate variables involved selecting the samples from various locations of the sample rolls and using air permeability measurements to select samples of similar structure.

Testing showed that the percent penetration values "at a particular flow rate with different spore-challenge concentrations are generally close to one another and apparently fall randomly around a common value ("Influence of Dispersion Concentration", 36)." The researchers concluded that at the predetermined flow rate "percent penetration was independent of flow rate." The conclusion is that, although some standards may require extremely high spore concentrations for microbial barrier testing, these results can be used to predict

the microbial barrier performance at lower “real-world” spore concentration rates (“Influence of Dispersion Concentration”, 37).

Affect of Material Structural Variations on Microbial Penetration Properties

Much of the research performed by Tallentire and Sinclair has suggested that a material’s microbial barrier properties are “dependent in part on its ability to act as a depth filter. (“Variations in Structure”, 57)” If this is accepted as true, there is the question as to how microbial penetration is affected by variations in a material’s structure. Manufacturing processes for medical-grade papers allow for a fairly consistent structure throughout a roll, but the very nature of Tyvek® makes structural consistency virtually impossible. In the article “Variations in Structure and Microbial Penetrability of Uncoated Spunbonded Polyolefin (Tyvek®),” Tallentire and Sinclair explored the effect a material’s structure had on its microbial filtration capabilities.

Tallentire and Sinclair tested both 1059B and 1073B uncoated Tyvek® grades. In an effort to minimize the effects of flow rate, they tested at four significantly different flows: 1, 6, 20, and 100 $cm^3\ min^{-1}\ cm^{-2}$. Their intent was to use air permeability and thickness measurements to characterize the various densities of Tyvek® samples. Then they subjected the characterized sample to microbial penetration testing to determine if a correlation existed. They tested 50 samples of each material, from various locations on the rolls, for both air permeability and microbial penetration.

They determined that both flow rate and thickness of a material have a “major role in determining microbial penetrability toward airborne bacterial spores

(“Variations in Structure”, 61).” They further conclude that the web structures for medical-grade Tyvek® and microbial penetrability “can be correlated.”

This information confirms that material structure is a dynamic variable when testing materials with respect to microbial penetrability and must be factored into the test design accordingly.

MATERIALS AND METHODS

Equipment

Preconditioning

Achieving the desired RH levels, 15%, 50%, and 90%, required construction of three temporary atmospheric chambers. These chambers were built using three 20 gallon aquariums (Figure 13) and various salt solutions. Silicon grease and fiberglass were used to form an air tight seal around the top of the three aquariums. With the temperature remaining constant at 23 ± 1 ºC (73.4 ± 2 ºF), it was determined that Lithium Chloride, Magnesium Nitrate, and Potassium Nitrate would be the saturated salt solutions required to achieve levels close to the, low (15%), nominal (50%), and high (90%) RH levels desired (Greenspan, 89). Sper Scientific and Oakton hygrometers were placed in the temporary environmental chambers and used to read the real-time RH and temperature values.

Table 2: Identification of salt solutions to relative humidity levels (Greenspan 89-93)

Saturated Salt Solution	**Relative Humidity % (at 20ºC)**
Lithium Chloride, LiCl	11.31 ± 0.31
Magnesium Nitrate, $Mg(NO_3)_2$	54.38 ± 0.23
Potassium Nitrate, KNO_3	94.62 ± 0.66

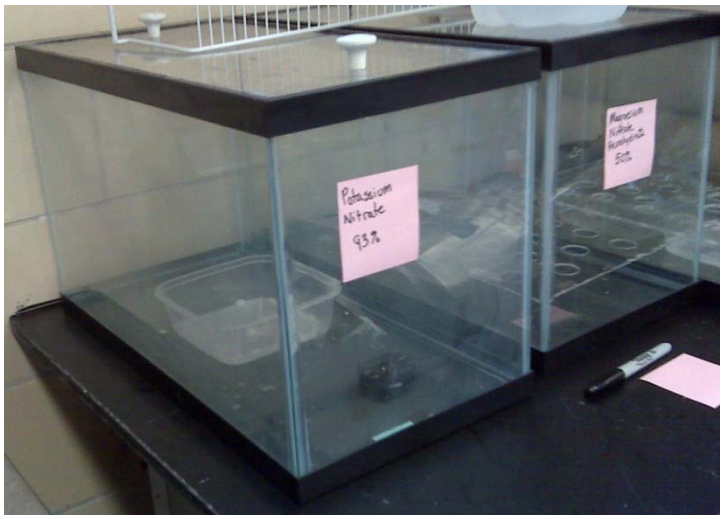


Figure 14: Conditioning chambers

Tie racks, purchased from Home Depot, were used to keep the samples separated and vertical during preconditioning, which would ensure that all surfaces of the sample were exposed to the environments moisture.

Testing

Testing primarily followed the ASTM F2638-07 test standard, which outlines a specific configuration of equipment for both dual and single particle counting systems. Since this test standard is relatively new, the DuPont facility in Richmond, Virginia is the only location in the world with the equipment arrangement required by ASTM F2638-07. Thus, it was necessary to conduct the conditioning and testing using the dual particle system at DuPont's Richmond facility.

The equipment is configured, as demonstrated in Figure 14, using a nebulizer, two particle counters, data logging system, manometer, pressure regulator, and a customized sample holding fixture. All flow readings were in direct engineering units, standard liters/minute.

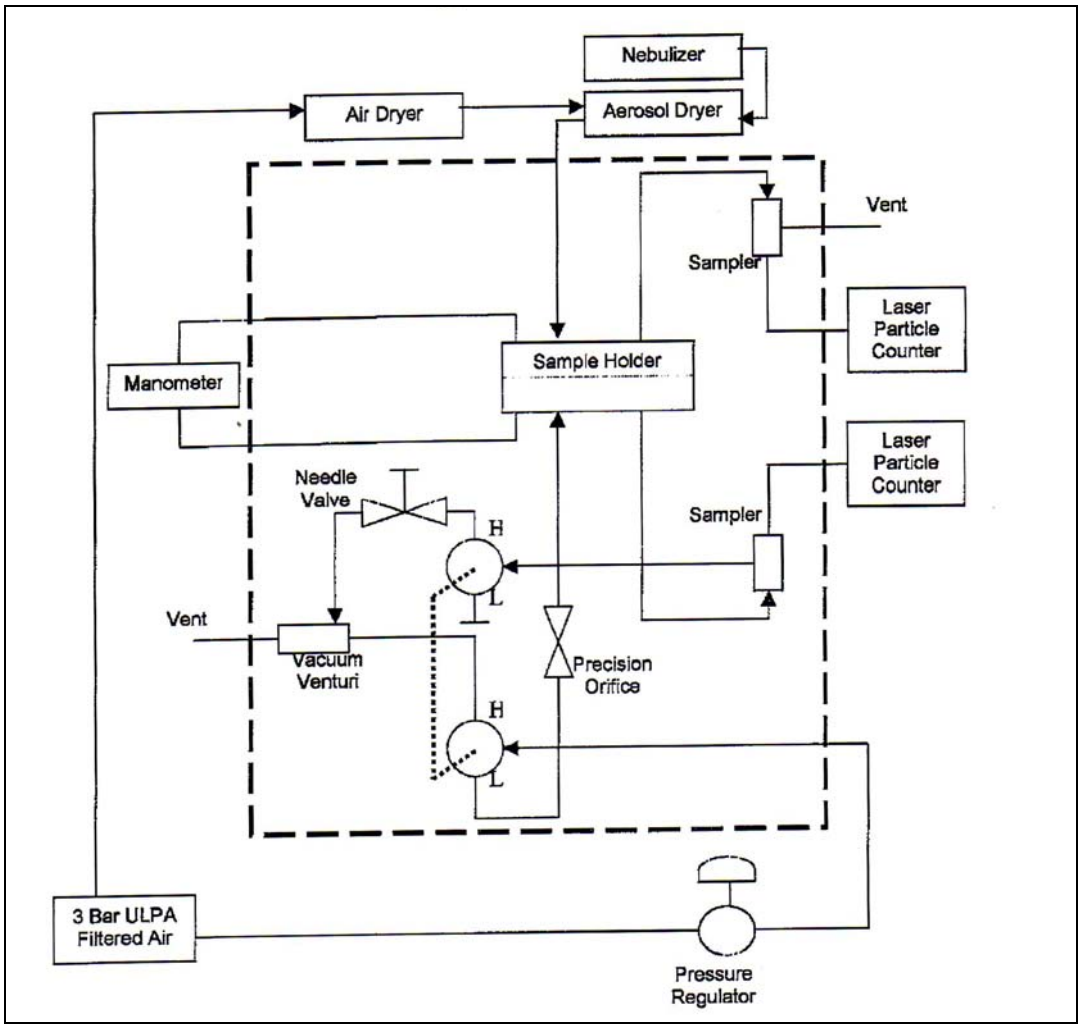


Figure 15: Equipment configuration for F2638-07 (ASTM F2638-07)

Nebulizer

A vertical nebulizer is used as the aerosol generator which disperses the particles from their liquid solution. Periodically, an ultra sonic cleaner, seen in Figure 15, is used in conjunction with the nebulizer to ensure the particles remain suspended within their liquid solution.

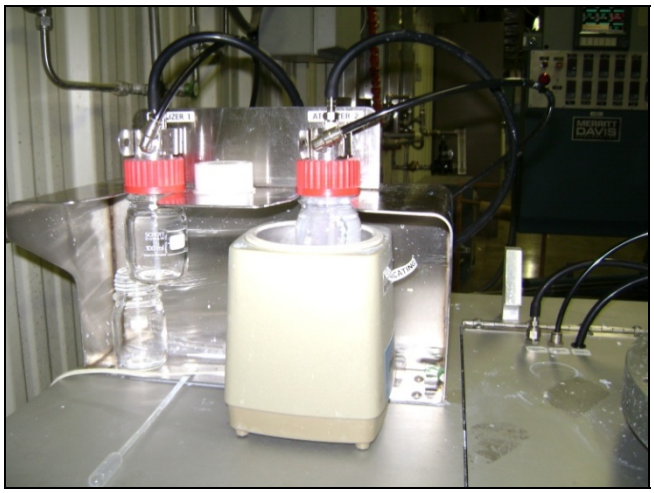


Figure 16: Nebulizer with ultrasonic agitation

Particle Counters

Method B of ASTM F2638-07, requires the use of two particle counting units, measuring the challenge and filtrate counts respectively. DuPont's system used two Lasair series Model 1003 particle counters from Particle Measuring Systems, Figure 16. This system is capable of counting and classifying particles that are 0.7 µm and 1.0µm in diameter.

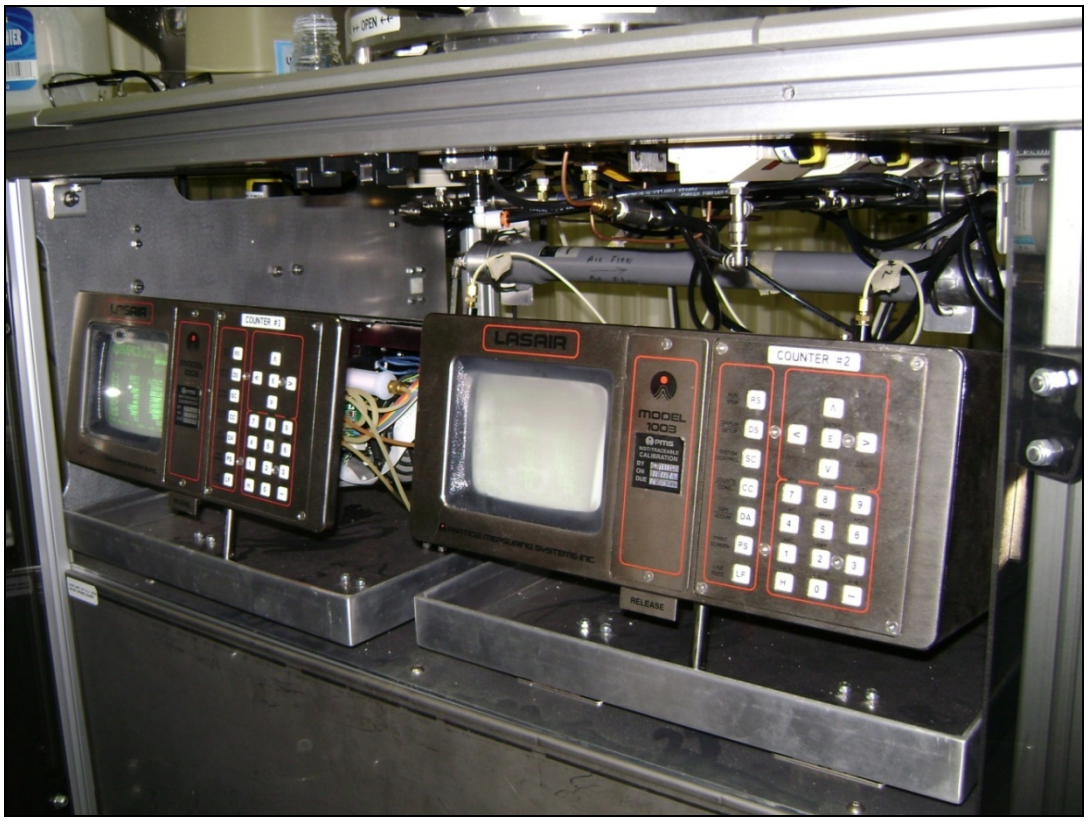


Figure 17: Dual particle counters (Lasair series)

Data Logging Software

DuPont has developed custom software that collects the elapsed test time (hours/minutes/seconds of the day), pressure differential (psig), total challenge particles (#), total filtrate particles (#). The software also calculates the flow rate (lpm) across the test sample, Figure 17. The system then records and graphs these measurements every six seconds throughout the duration of the test, Figure 18.

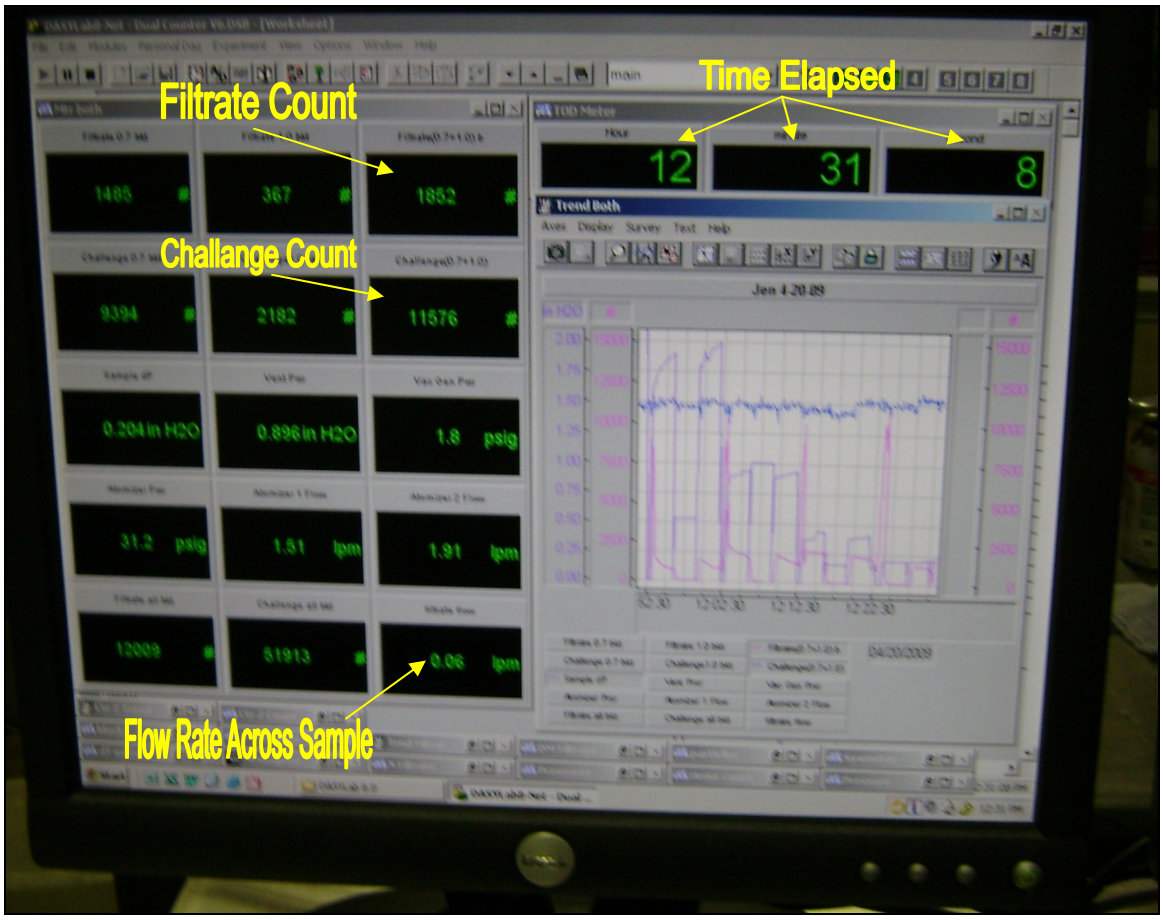


Figure 18: Data tracking software interface

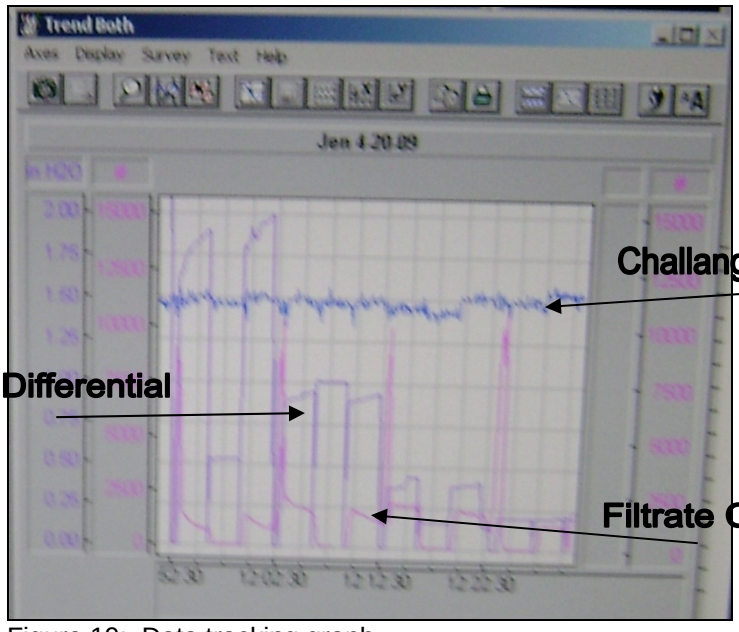


Figure 19: Data tracking graph

Manometer

The manometer is used to measure the pressure differential across the sample. Precision of the manometer is critical and it must maintain a minimum range of 0 to 5 cm WC with an accuracy of 0.005cm WC (ASTM 2638-07). Readouts from the manometer were displayed on the data logging screen and adjustments were made to pressure using a series of controls from the equipment control panel, Figure 19.

Figure 20: System control panel

Customized Sample Holder

Samples are held by a custom sample holder, which exposes a 100cm diameter area of the sample to the desired particulate challenge. The holder consists of two assemblies that deliver a uniform flow of aerosol across the test

specimen (ASTM 2638-07: 7.1.1). A dimensioned drawing of the sample holder is available in ASTM F2638-07: Figure 2.

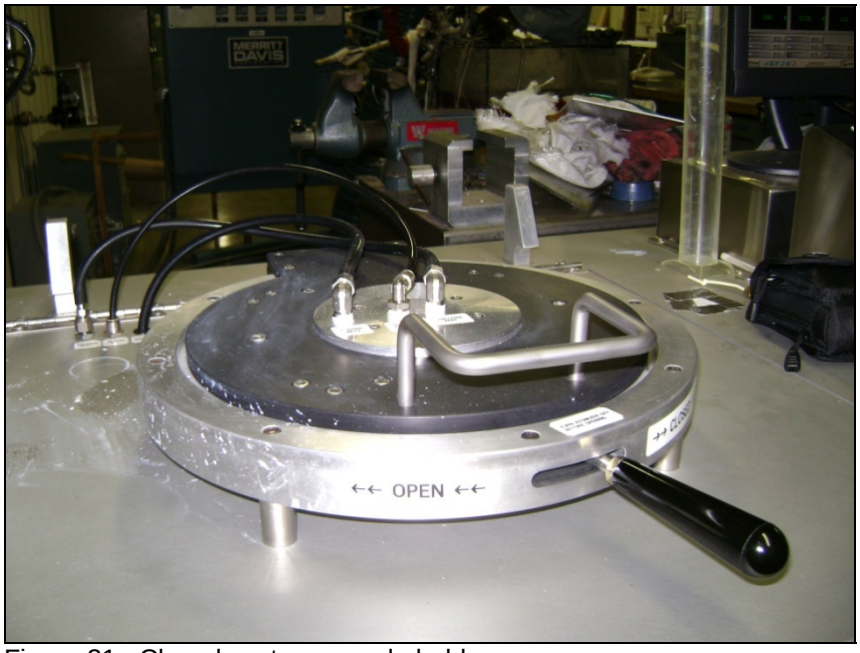


Figure 21: Closed custom sample holder

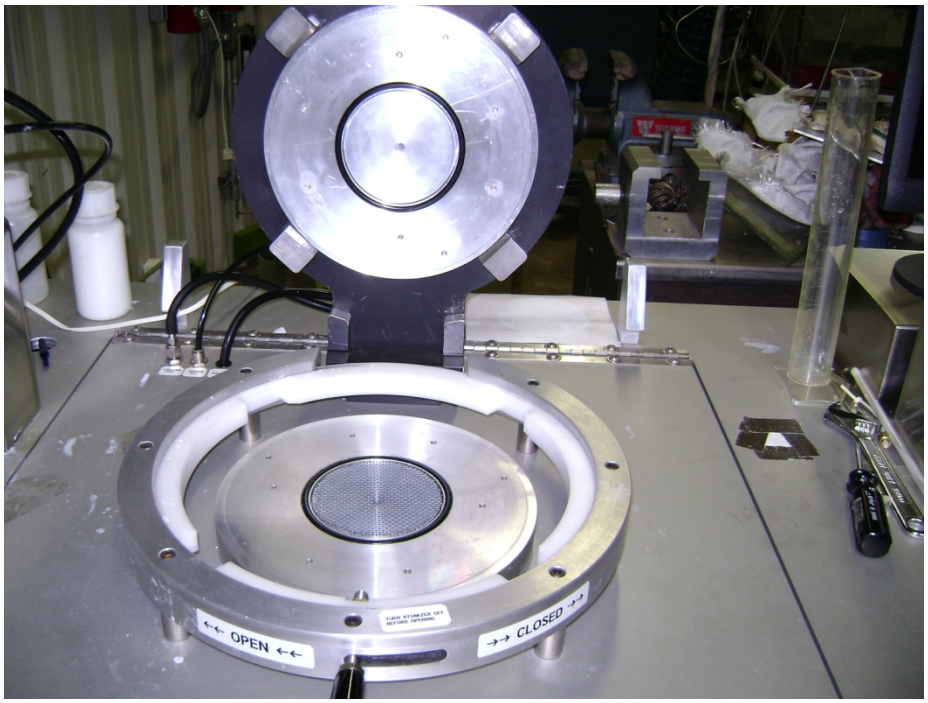


Figure 22: Opened custom sample holder

Materials

Samples

Tyvek® and medical-grade papers were chosen for the testing due to their prominent use in medical device packaging, while Ovantex® was chosen because of its recent introduction to the medical packaging market and lack of research available about its properties. The materials tested include uncoated Tyvek® 1073B, coated Tyvek® 1073B, dot coated Ovantex®, and coated 55 pound latex impregnated medical grade paper. Since sealability limits the use of uncoated medical-grade paper in the medical industry, testing was only performed on a coated form. Fifty-five pound latex impregnated medical-grade paper was chosen because it has the highest sales volume for medical-grade papers in the medical device industry (S. Belmonte, Perfecseal, personal communication, 2009). Given that that Tyvek® is used frequently as both an uncoated and coated substrate, both structures were chosen for testing. The coatings on both the Tyvek® 1073B and the medical-grade papers were CR27 from Perfecseal, which is the most common coating sold in the medical device industry (S. Belmonte, Perfecseal, personal communication, 2009). Ovantex® is coated with a dot coating that is exclusive to Oliver® Medical.

Particulate

As outlined by ASTM F2638-07, the particulate used in testing was made of polystyrene latex (PSL) particles measuring at 1µm in diameter. The product used was product 5100A Duke Scientific.

Defining Variables

Preconditioning-Humidity

The test was aimed at challenging the microbial penetration of materials at extreme and nominal relative humidity levels. Because this testing was focused on relative humidity levels, the temperature was set at standard lab conditions, 23 ± 1 ºC (73.4 ± 2 ºF), and testing humidity levels were chosen in accordance to ASTM D4332-01: Standard Practice for Conditioning Containers, Packages, or Packaging Components for Testing. To perform this test, samples were conditioned at three RH levels: low, nominal, and high. The D4332-01 standard, states that relative humidity of 50 ± 2% is a standard conditioning atmosphere, so that level was selected for the nominal value for this testing. Extreme RH levels were similarly determined using Table 1 of the D4332 test standard. This table refers to a RH of 90 ± 5% as a “high humidity” or “tropical” environment, and a RH of 15 ± 2% as a “desert” or “low humidity” condition. Test samples were exposed in the environmental chambers, discussed in Equipment section, for a period of at least 48 hours.

Testing-Particulate Challenge

A concentration level of 10,000 particles per cc was selected. As discussed in literature review, over challenging the samples was not a concern, since microbial penetration rates are independent of dispersion concentration levels.

Testing-Flow Rates

Testing protocol followed ASTM 2638-07 procedure for Method B Dual Particle Counter (Section 11.2) with a slight variance; samples were only tested

for a single flow rate. This decision was made to reduce the likelihood of drying out the preconditioned material sample and to reduce the potential for particle blockage due to a “particle packed” sample.

Since all previous studies concealed the material identification, it was necessary to perform the ASTM 2638-07 test standard on each of the unconditioned materials to approximate the maximum particle penetration flow rate (P_{max}) value. Since the P_{max} value is the point of optimum particle penetration, it is critical to incorporate these values during testing to ensure the worst-case scenario is being tested. Once this value was determined, the material specific flow rate range was selected for testing.

Each material was run following the ASTM 2638-07 test standard with the challenge concentration levels at 10,000 spores dm^{-3}. The only deviation from the ASTM test standard was that the test was begun with at the minimum flow rate, 0.03 slpm, as opposed to beginning at a higher pressure differential and flow rate. The flow rate was then doubled until a peak was observed in the filtered particle counts, followed by a continuous decrease. With this information, it was possible to approximate the P_{max} by calculating the percent of particle penetration and graphing it verses the flow rate, from the test output (Figure 23). Previous research in this area presented graphs in log-log format. This research used standard graphing, however log-log graphs can be found in the appendix.

$$\%\ Particle\ Penetration = \frac{\#\ Filtrate\ Particles}{\#\ Challange\ Particles} x\ 100 \qquad \text{(Equation 4)}$$

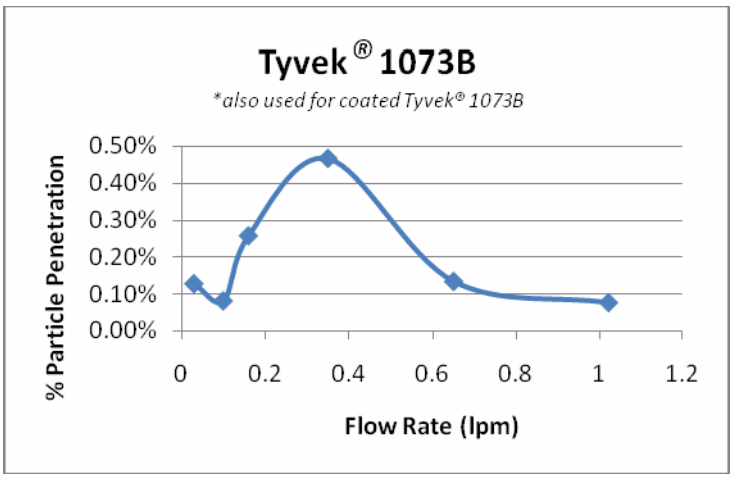


Figure 23: Optimum flow rate for microbial penetration (additional material graphs in the appendix)

Although the previous research done calculating P_{max} values hid the identity of the materials tested, most of the presented data shows the P_{max} values ranging within 10^{-2} and 10^{-1} $dm^3min^{-1}cm^{-2}$. As is visible from the log-log graphs, in the appendix, this research also determined the materials' P_{max} values to be within this range. This close replication from previous research provided additional confidence in the test administration. The information was then used to select the material specific flow rates for the relative humidity tests, Table 3. Flow rates selected for the Tyvek® 1073B, were duplicated for the coated Tyvek®, 1073B CR27, because there were no filtrate particles observed for the coated Tyvek®.

Table 3: Samples and flow rates chosen for testing

Sample	**Flow Rates (lpm)**
1073B	0.06, 0.12, 0.24, 0.48, 0.96
1073B CR27	0.06, 0.12, 0.24, 0.48, 0.96
Ovantex®	0.03, 0.06, 0.12, 0.24, 0.48
55# latex impregnated paper	0.03, 0.06, 0.12, 0.24, 0.48

Statistical Randomization

Since complete randomization is ideal for most experiments, testing was randomized using a complete block design. In a complete block design, each flow rate is applied to individual samples that are selected at random within each run for each round (Larget, 2)." The test was comprised of three rounds, where 60 samples were tested in each round. Each round was comprised of 5 runs at various increasing or decreasing flow rates. The 5 runs within each round alternated between ascending and descending flow rate ranges. There were three relative humidity changes within each flow rate change in the run. Relative humidity samples were completely random. Table 4, below, is designed to provide a visual representation of one round for this test.

Table 4: Complete block design, example of round

Round 1	RUN 1	0.03			0.06			0.12			0.24			0.48			0.96		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R
	RUN 2	0.96			0.48			0.24			0.12			0.06			0.03		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R
	RUN 3	0.03			0.06			0.12			0.24			0.48			0.96		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R
	RUN 4	0.96			0.48			0.24			0.12			0.06			0.03		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R
	RUN 5	0.03			0.06			0.12			0.24			0.48			0.96		
		R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R

Test Method

Sample Preconditioning

- One hundred and eighty 140mm square samples, 45 samples for each of the four materials, were cut using a 140mm square template.
- In an effort to characterize the materials being tested, thickness and weight measurements were recorded for all the material samples prior to conditioning.
- The samples were then equally separated, 15 sample of each material, into the three RH chambers for preconditioning, for a minimum of 48 hours. Hygrometers were used to provide a “real-time” readout of the chambers RH levels.

Testing Preparation

- Following ASTM 2638-07 test method B, a 200-8000 particles/ml solution was made using the polystyrene latex particles and distilled water.
- The particulate solution was connected to the nebulizers, and all the machine components were activated.
- Once the particle count readouts stabilized, adjustments were made to the flow through the nebulizer to adjust for a 10,000 particles per dm^{-3} concentration.
- Once the challenge and filtrate particle counters stabilized and were within 3% variation of 10,000 particles per dm^{-3}, testing began.

Testing

- Testing was performed by selecting material samples according to the complete block experimental design discussed previously.
- Samples were removed from the relative humidity chamber and weighed on an analytical balance.
- Weights were recorded and the samples were secured into the sample holder for the duration of the test, 3 minutes. Two minutes were used to stabilize air flow and particulate levels, and one minute for the particulate challenge.
- Time range for the testing was recorded, so data could be extracted for analysis.
- Samples were removed from the sample holder and weighed.
- Post-test weights were recorded.
- The process was replicated for all 180 samples tested following the complete block experiment design

Characterizing the Samples

The initial data collected to characterize the material samples shows that all four materials remain relatively consistent in weight. However as expected, due to the structural variations, the Tyvek® 1073B and 1073B CR27 are highly variable with regard to thickness.

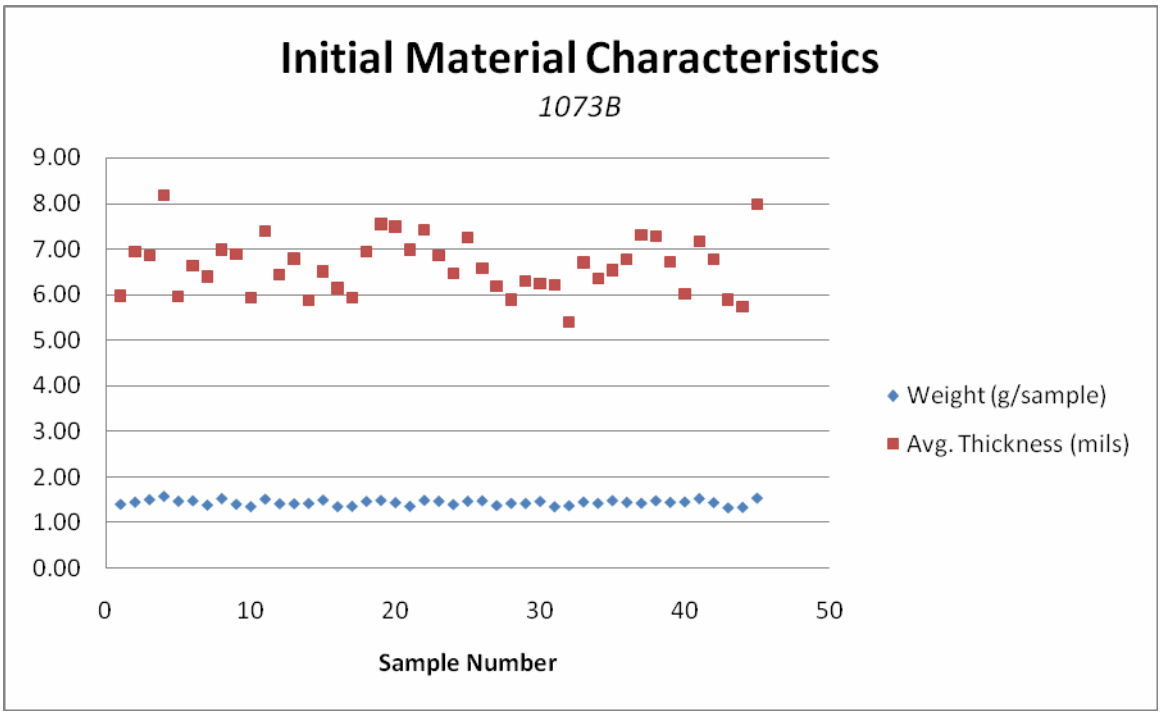


Figure 24: Initial material characteristics- Tyvek® 1073B uncoated

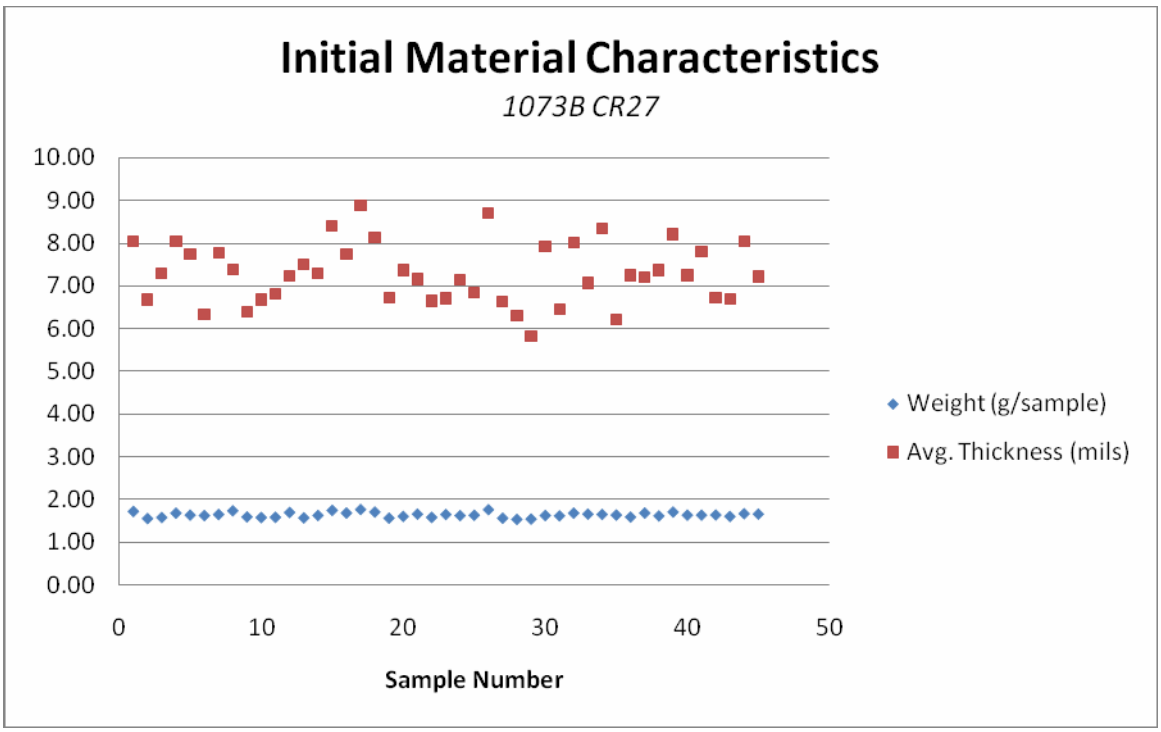


Figure 25: Initial material characteristics- Tyvek® 1073B coated

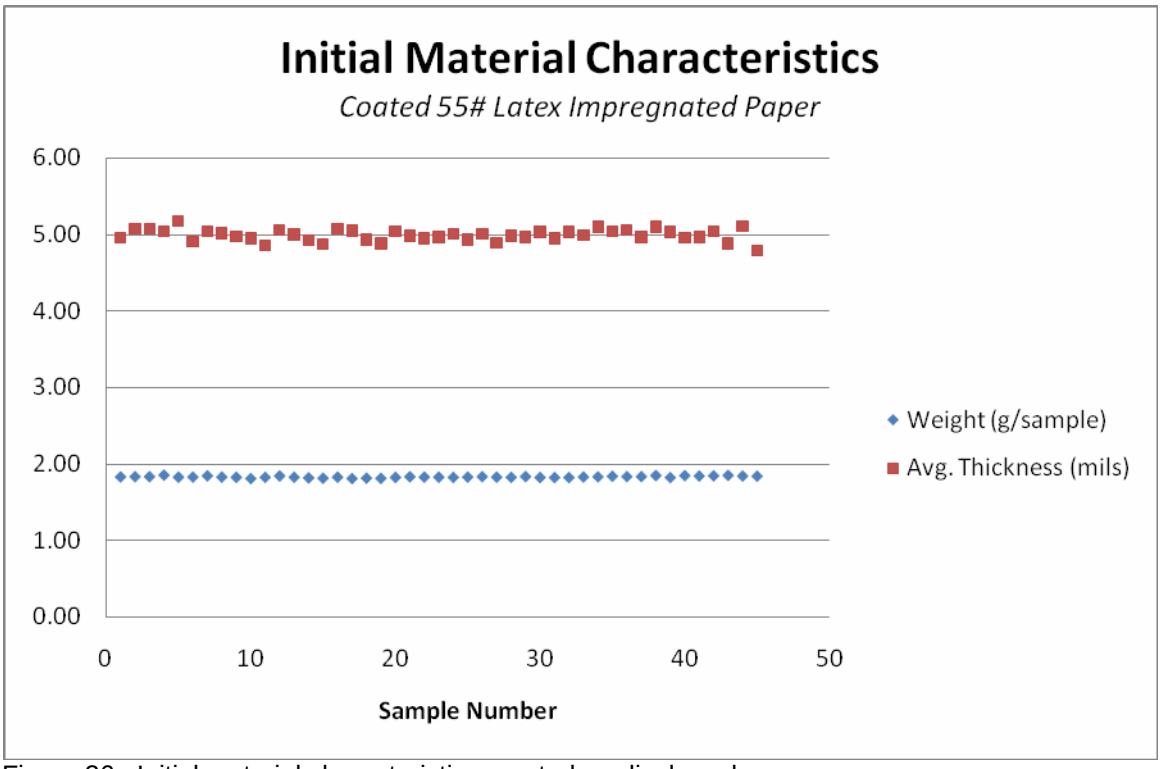


Figure 26: Initial material characteristics- coated medical-grade paper

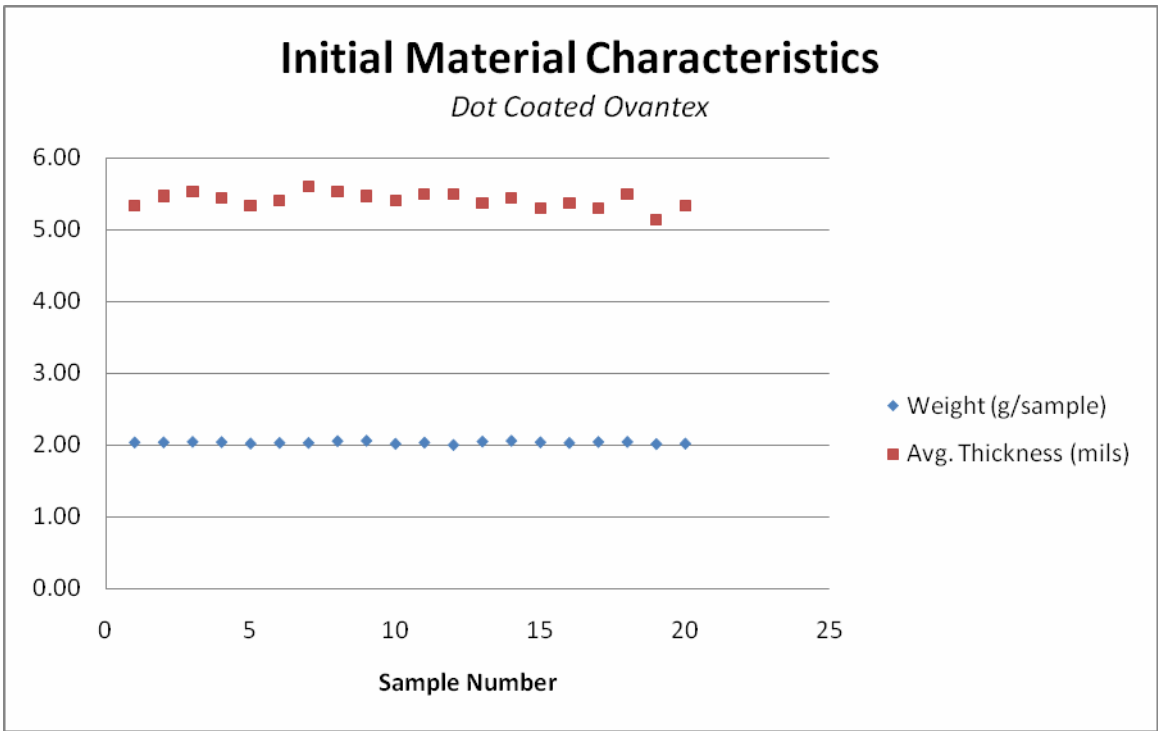


Figure 27: Initial material characteristics- dot coated Ovantex

Table 5: Observed measurements for characterizing the samples

	Avg Weight (g)	Std Dev Weight	Avg Thickness (mils)	Std Dev Thickness	Basis Weight (g/m^2)
1073B	1.434	0.061	6.641	0.612	0.073
1073B CR27	1.650	0.058	7.296	0.709	0.084
Coated 55# Paper	1.819	0.011	4.988	0.075	0.092
Coated Ovantex	2.03	0.01	5.41	0.11	0.10

Conditioning Effects

Since salt solutions were used to maintain the relative humidity levels in the environmental chambers during preconditioning, slight variations from the planned relative humidity levels were expected. The following table provides the data collected from the hygrometer during the designated day of testing.

Table 6: Actual recorded RH levels during testing

	Day 1	Day 2
Low	19%	21%
Nominal	51%	52%
High	87%	86%

Although initial weights were measured at an undetermined relative humidity level, post-conditioning weights can be used to generally understand the materials water retention capabilities. Since Tyvek® is manufactured from 100% HDPE, it was not expected to respond significantly to changes in relative humidity. In studying Figures 28 and 30, neither the Tyvek® 1073B nor the coated Tyvek® 1073B CR27 showed any consistent response to RH levels. This is confirmed by Figures 29 and 31, which show the average moisture change and standard deviations with respect to relative humidity levels. It is, however, interesting to see that although the responses to RH levels are inconsistent, both the coated and uncoated structures do show moisture gains and losses. This was an unexpected phenomenon that warrants further research. It is possible that the observed moisture variation is attributed to the variation in densities throughout the Tyvek® structure. However in this experiment, samples were not individually marked; making it impossible for this data to determine the densities relevance to moisture gains or losses within Tyvek® 1073B coated and uncoated.

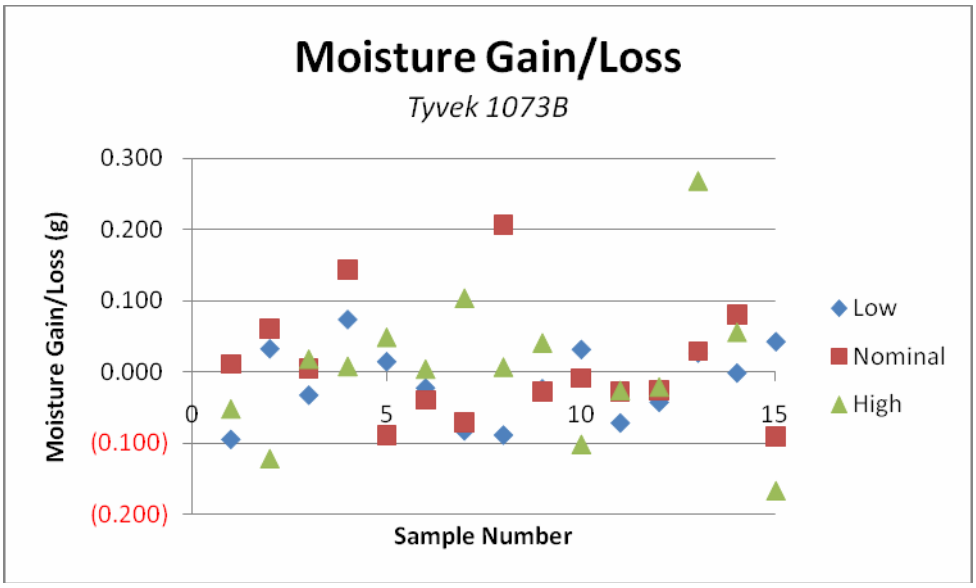


Figure 28: Moisture gain/loss-Tyvek® 1073B uncoated

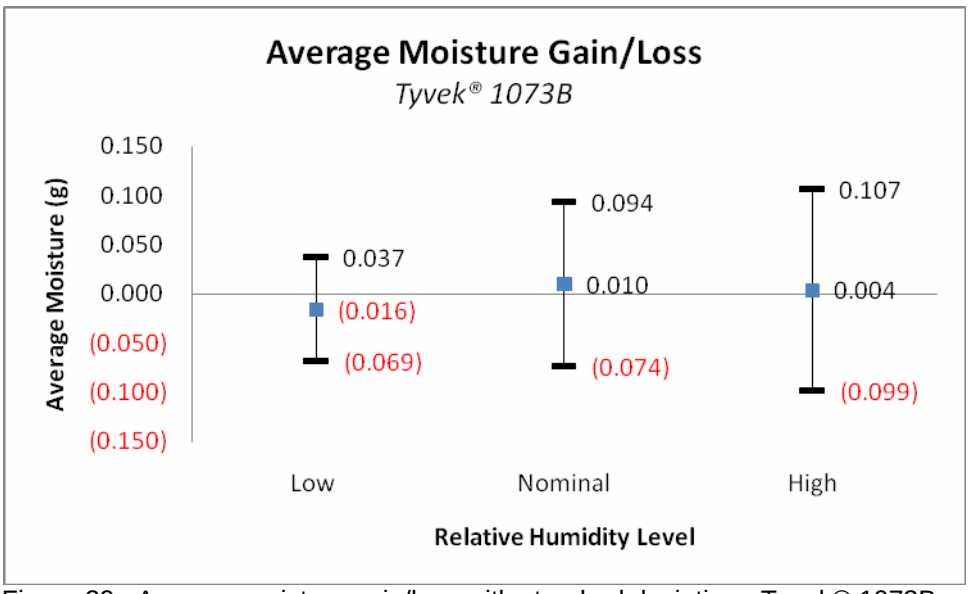


Figure 29: Average moisture gain/loss with standard deviations-Tyvek® 1073B uncoated

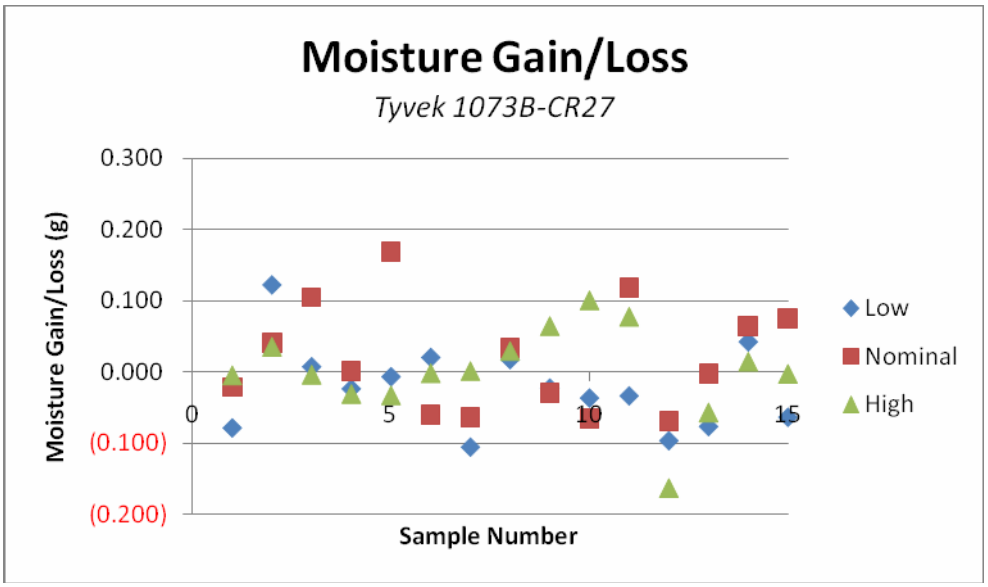


Figure 30: Moisture gain/loss- Tyvek® 1073B coated

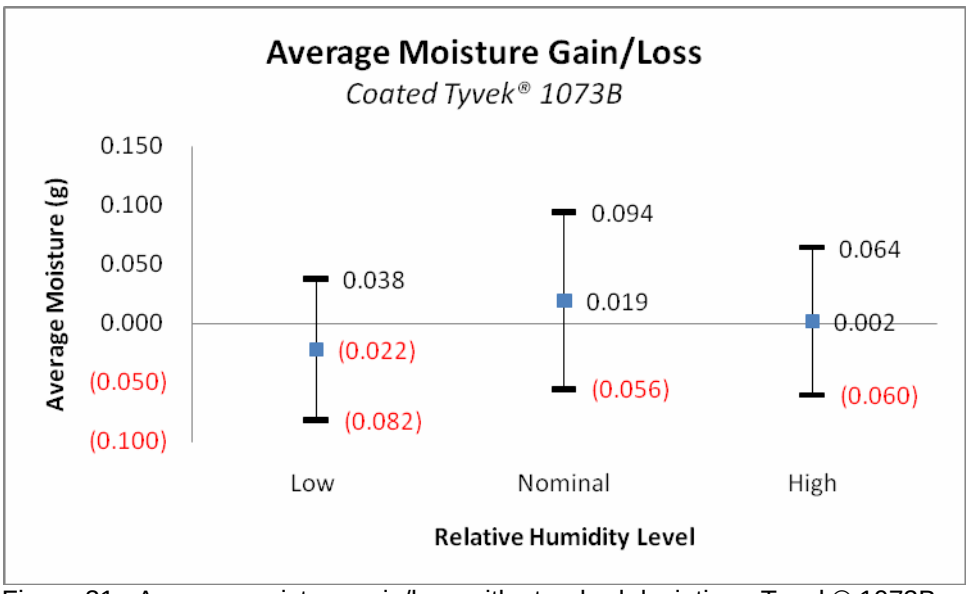


Figure 31: Average moisture gain/loss with standard deviations-Tyvek® 1073B coated

As expected, the materials containing cellulose fibers, Ovantex and 55# Paper, demonstrated fairly consistent responses at each of the tested relative humidity levels. The change in consistency, with respect to moisture, change at various relative humidity levels, from the Tyvek® 1073B structures, Figures 28-31, and the Ovantex® and medical-grade paper, Figures 32-35, can most likely be attributed to the presence and prominence of cellulose fibers in both the

Ovantex® and medical-grade paper structures. These cellulose fibers are more likely, than HDPE, to absorb/desorb the moisture in the RH chambers, which allows the materials to show more consistent responses to the change in RH levels.

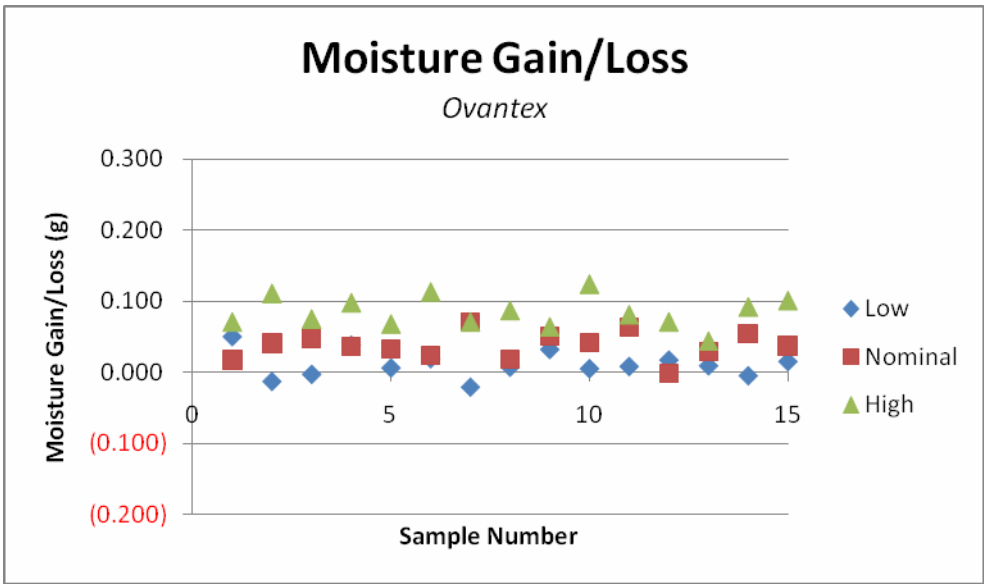


Figure 32: Moisture gain/loss- dot coated Ovantex

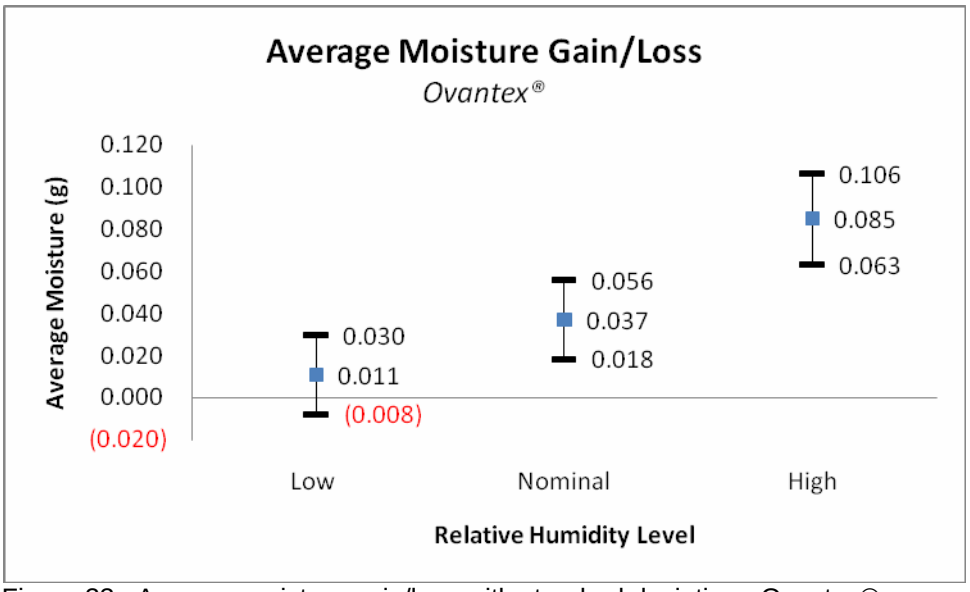


Figure 33: Average moisture gain/loss with standard deviations-Ovantex®

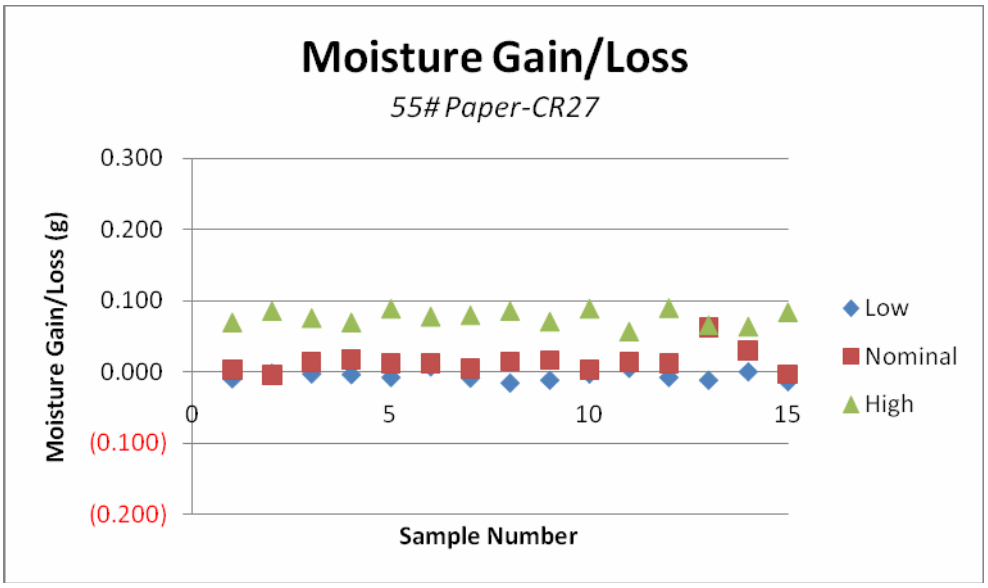


Figure 34: Moisture gain/loss- coated medical-grade paper

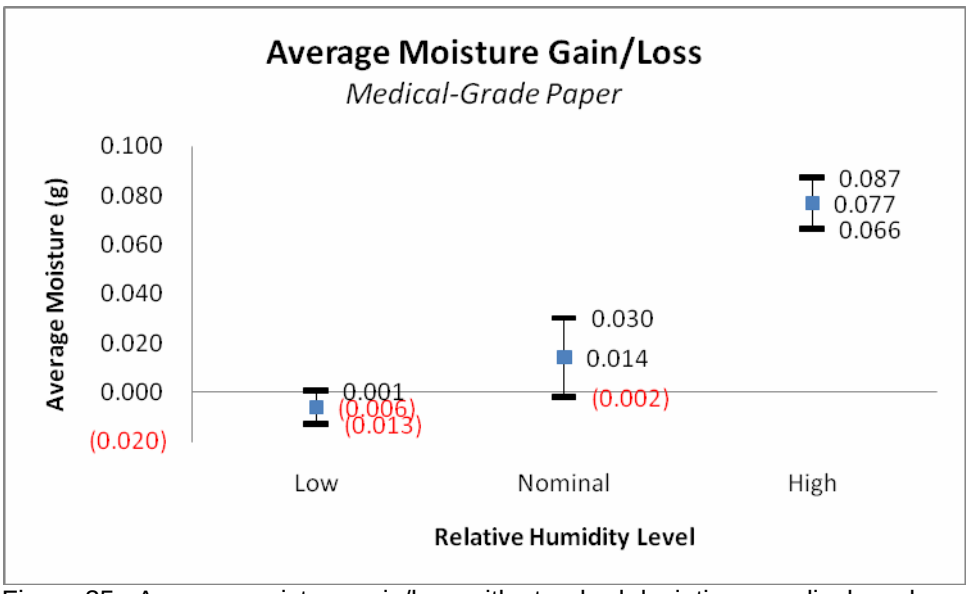


Figure 35: Average moisture gain/loss with standard deviations-medical-grade paper

Microbial Penetration Test Results

The average percent penetration through each material was plotted in Excel and analyzed. Figures 38 and 39 show distinct microbial penetration vs. RH level curves with values varying as RH levels changed. These observations led to the expectation that RH levels did significantly effect the microbial penetration for both the dot coated Ovantex and the coated 55# paper materials.

Figures 36 and 37 for the 1073B and coated 1073B CR27, respectively, demonstrated much variability in the microbial penetration data at very low penetration percentages and at all RH levels, which led to a hypothesis that neither the coated nor uncoated Tyvek® 1073B's microbial penetration rate was significantly dependent on RH levels. Although the results show in Figures 36 and 37 demonstrate a statistical difference, at the penetration percentages observed; the results do not show a practical significant difference. Variations shown by these graphs represent actual particle penetration variations of approximately ± 2 – 10 particles.

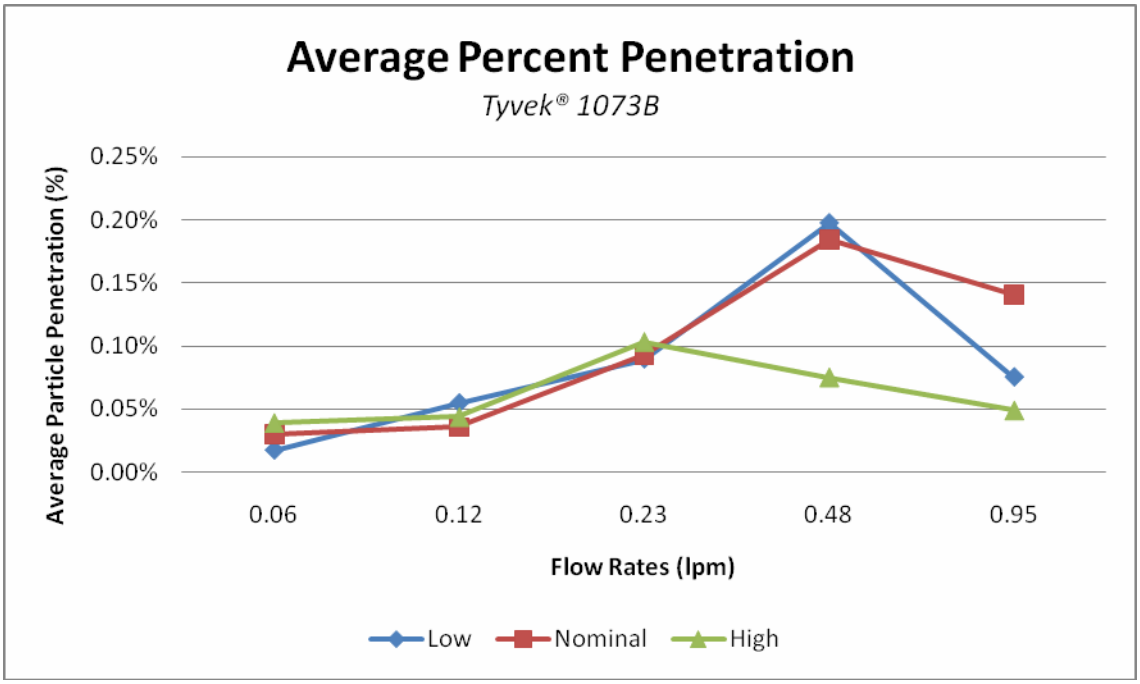


Figure 36: Average percent microbial penetration- Tyvek® 1073B uncoated

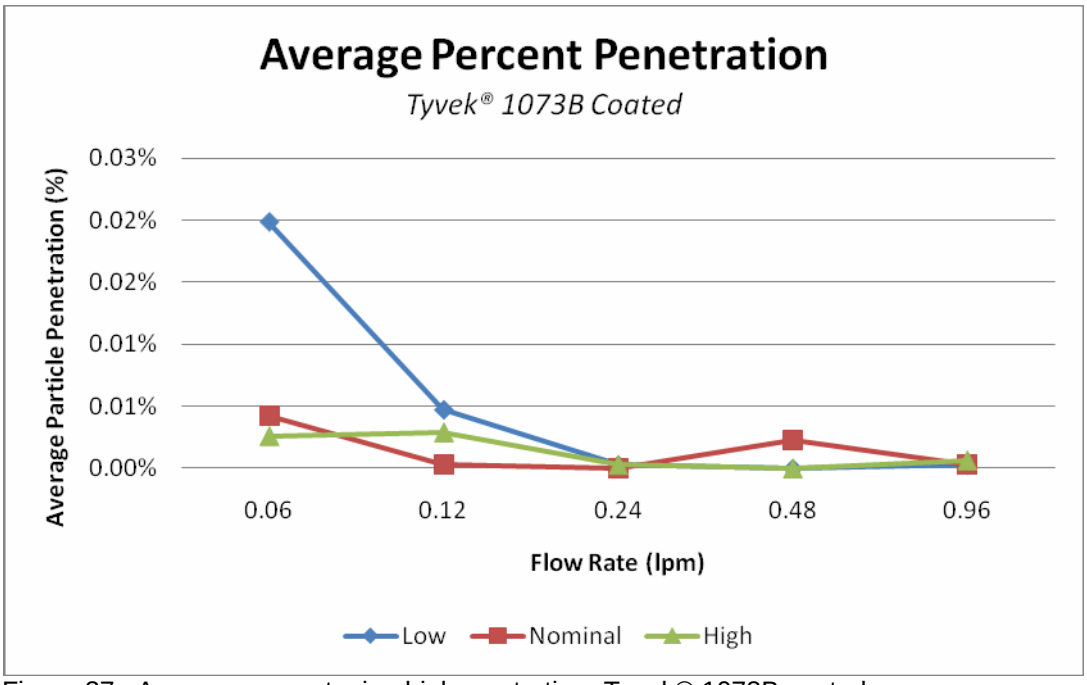


Figure 37: Average percent microbial penetration- Tyvek® 1073B coated

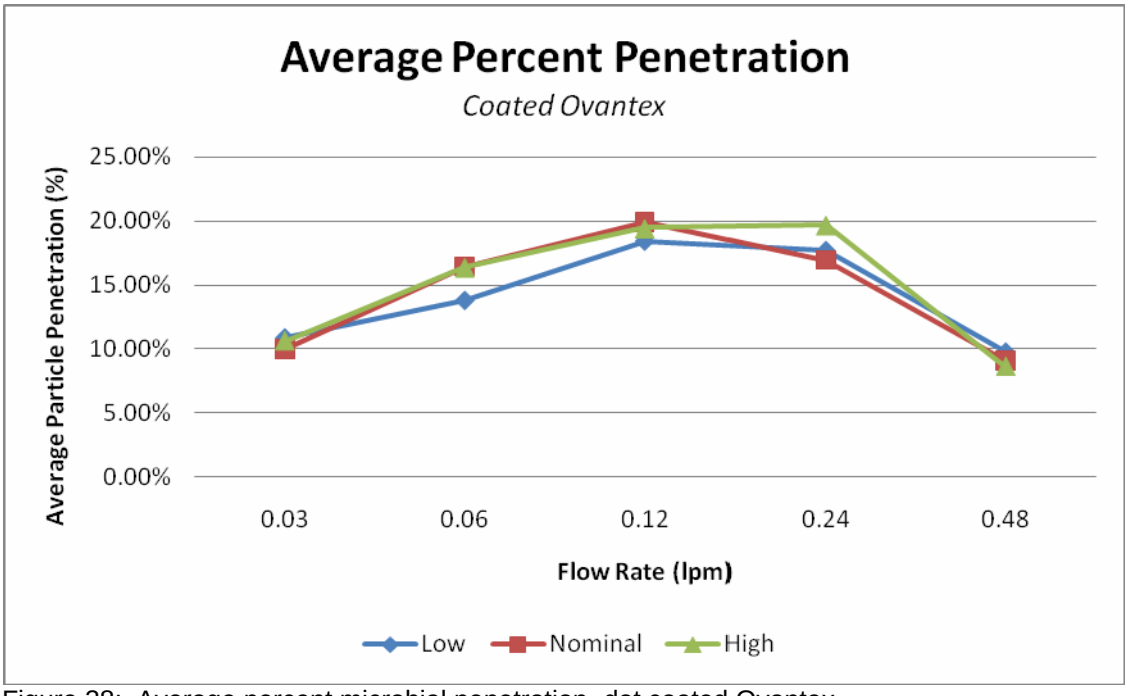


Figure 38: Average percent microbial penetration- dot coated Ovantex

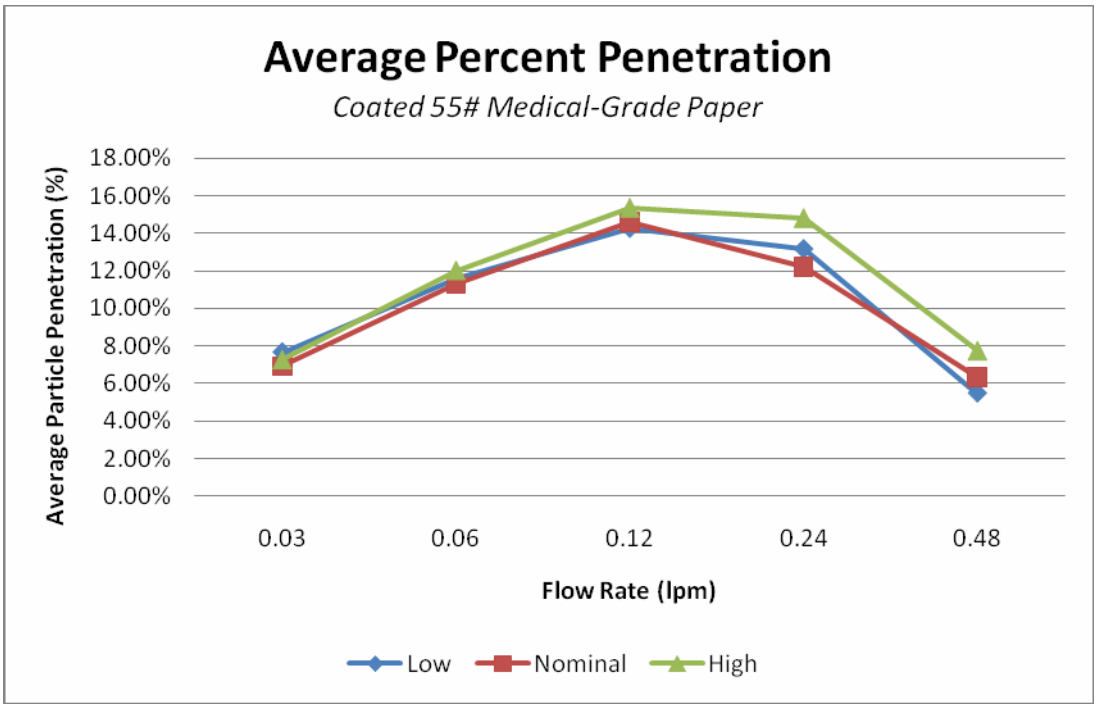


Figure 39: Average percent microbial penetration- coated medical-grade paper

Using Statistical Analysis Software (SAS) to perform a General Linear Model (GLM), these hypotheses were tested for statistical significance. In an effort to achieve a high degree of confidence in the test results, a statistical significance was selected as a p-value of 0.05. This means that, using a GLM, if the p-value is less than or equal to 0.05, then 95% of the time a correlation exists between the two variables being tested. "The GLM statistical procedure uses the method of least squares to fit general linear models (SAS website)."

The GLM analysis concluded, with a p-value greater than 0.05, that for each material, that the flow rate through the sample and relative humidity levels, affect the material's microbial penetration rates independently of one another, Table 7. This means that the two dynamic variables of the test, flow rate and RH level, are independent of one another. Since they are independent, further analysis can be done to test both variables for their relationship to the microbial

penetration properties. If these variables had been found dependent on one another, it would be impossible to determine whether flow rate or RH levels affected the microbial penetration properties of the sample.

Using the same GLM analysis techniques, a study was performed with respect to the two dynamic test variables and microbial penetration properties. Table 7 demonstrates the conclusion that the flow rate across the sample was only found to be significant, p=<0.0001, for the medical-grade paper and Ovantex. Relative humidity was found to have a significant effect, p=0.0016, on the microbial penetration properties for medical-grade paper.

Table 7: SAS generated p-values

	P-value flow rate and RH vs. microbial penetration	P-value flow rate vs. microbial penetration	P-value RH vs. microbial penetration
1073B	0.8929	0.0948	0.4743
1073B CR27	0.4574	0.4265	0.3817
55# Paper	0.1068	<0.0001	0.0016
Ovantex	0.2498	<0.0001	0.3268

Discussion

Flow rate results found in the medical-grade paper and the Ovantex were expected as earlier research has observed similar occurrences (“A Discriminating Method”, 241). This earlier research indicates a significance (p=0.05) of flow rate on microbial penetration for several materials. The current research shows a significance, p=0.10, between the flow rate and particle penetration for the uncoated Tyvek® 1073B. Further research would be necessary to determine whether it was the differences in test methods or the known structural variations in Tyvek® that caused the slight change in confidence for the flow rate and microbial barrier relationship.

It is recommended that future research characterizes the structural densities and porosities for individual Tyvek® samples. This characterization will make it possible to determine if observed inconsistencies or statistically insignificant findings are due to actual test parameters or variations in the materials' structure.

Since little research has been published on the effects of coating on Tyvek®, the findings of this research are interesting. This research suggests that the addition of Perfecseal's CR27 coating to Tyvek® 1073B significantly decreases the impact flow rate has with respect to microbial penetration. Future research examining various coatings with respect to their impact on microbial penetration and their impact on properties strongly related to microbial penetration (i.e. flow rate) could prove to be very beneficial research. Additionally, research using antimicrobial coatings, such as chitosan coatings, could aid porous materials with microbial barrier properties.

Because the cellulose fibers that form paper are susceptible to moisture, it was not surprising to find that relative humidity had a significant effect with regards to the microbial penetration thru medical-grade paper. It was interesting that the Ovantex, which also contains cellulose fibers, showed no significant relationship between microbial penetration and RH levels.

Since the exact makeup of Ovantex is unknown, it is impractical to make suggestions with regards to the effect of cellulose fibers and RH have on microbial barrier properties, however much research can be performed in this area. It would be interesting to determine what types of fibers are most

susceptible to changes in RH levels, and what percentage of cellulose fibers in a material structure compromise its microbial barrier properties. It would also be interesting to research how materials containing cellulose fibers react to microbes over time. Could microbes with a food source (cellulose fibers and water) eventually penetrate a package by "eating through" the material?

Conclusion

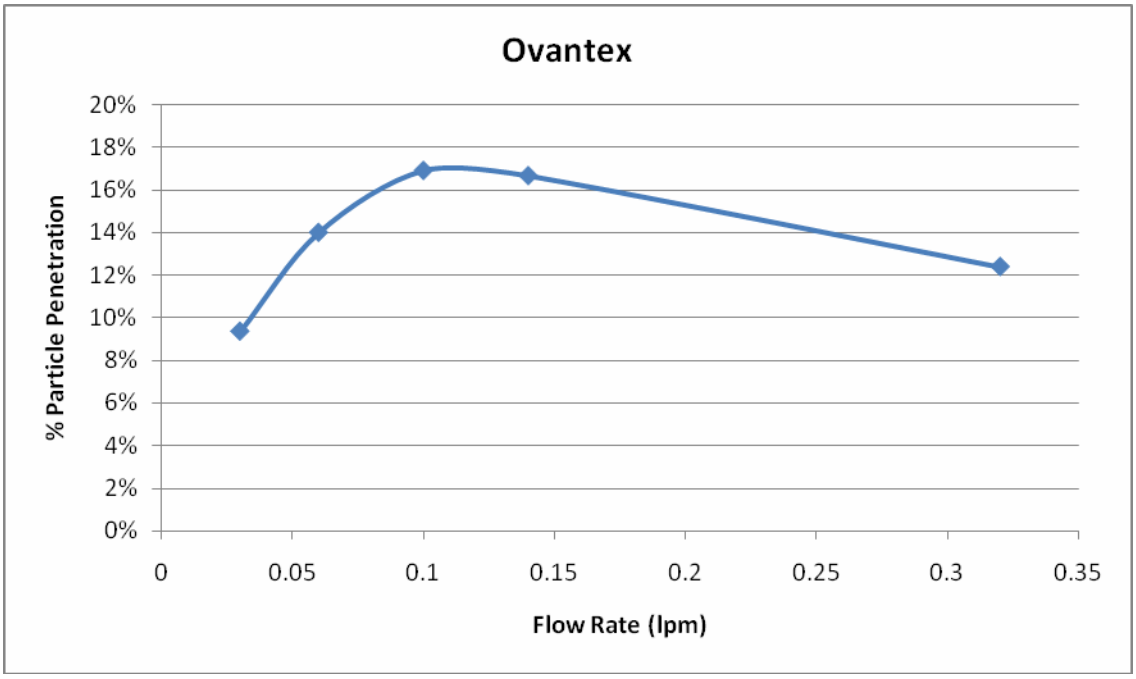
Relative humidity levels have a significant effect on the particle penetration levels for the medical-grade paper tested, 55 pound latex impregnated paper coated with Perfecseal's CR27 coating. Manufacturers planning to package using medical-grade paper should be concerned with the distribution and storage relative humidity levels for their products, because as previously stated, ISO 11607 requires manufacturers to ensure the sterility of their product from production until point-of-use.

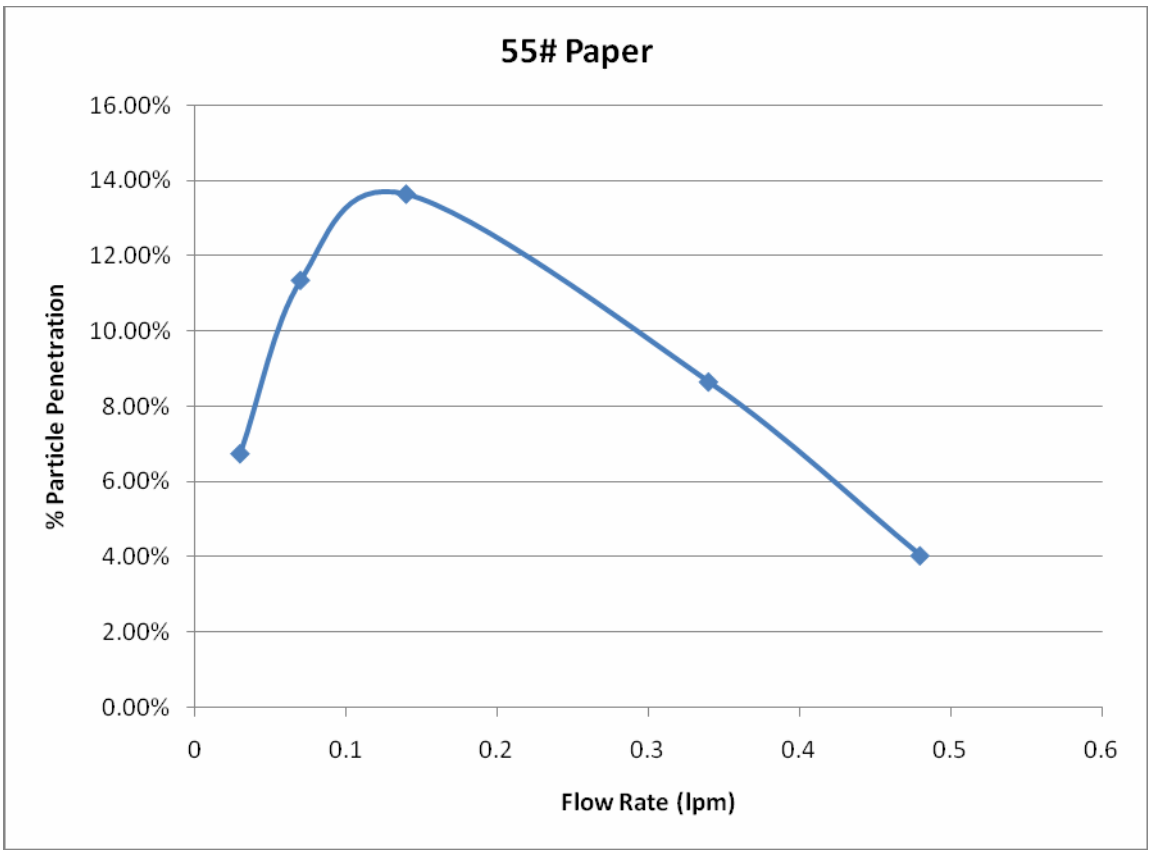
Relative humidity levels do not appear to have any effect on the particle penetration levels for the dot coated Ovantex, by Oliver® Medical, or the coated and uncoated 1073B Tyvek®, by DuPont.

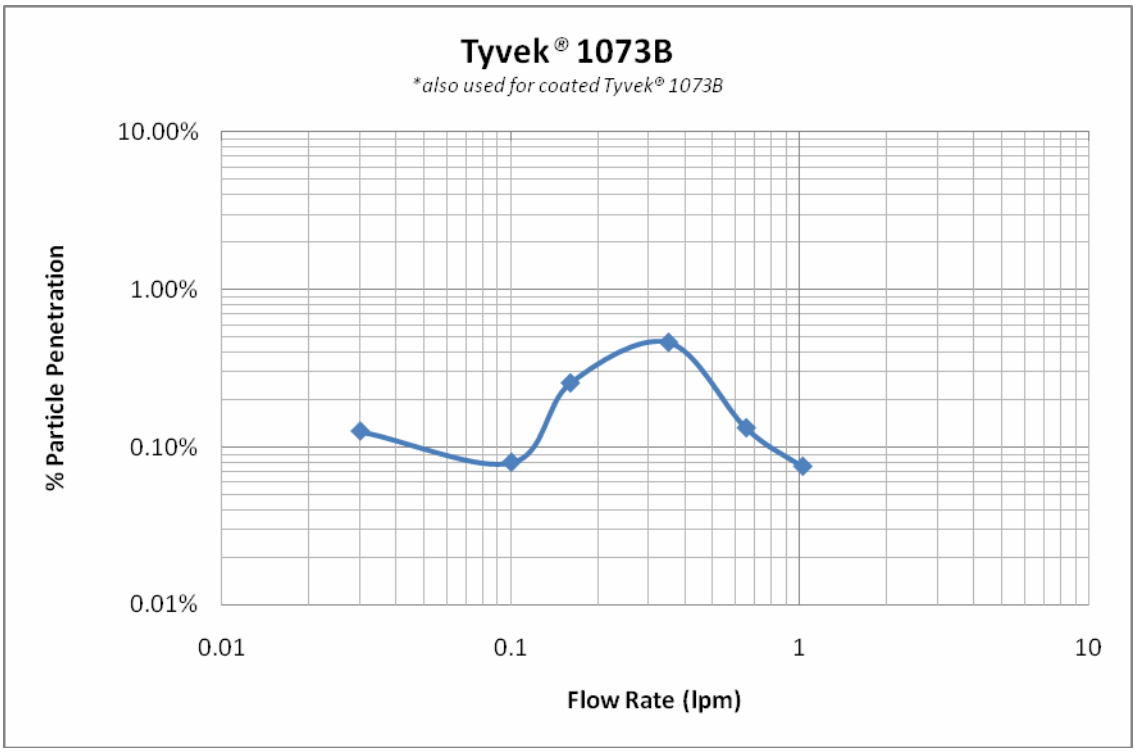
Table 8: Summary of conclusions

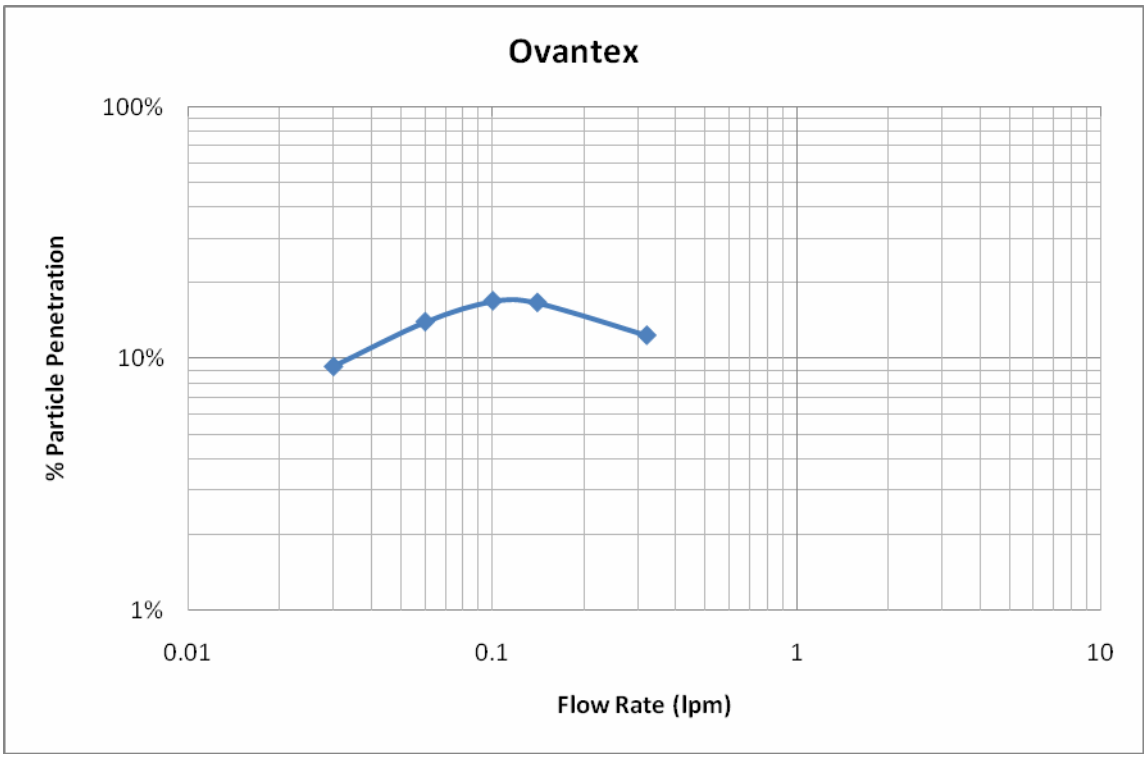
Material Tested	Does flow rate effect microbial penetration? (95% confidence)	Does RH level effect microbial penetration? (95% confidence)
Tyvek® 1073B	No	No
coated Tyvek® 1073B	No	No
dot coated Ovantex	Yes	No
coated 55# medical-grade paper	Yes	Yes

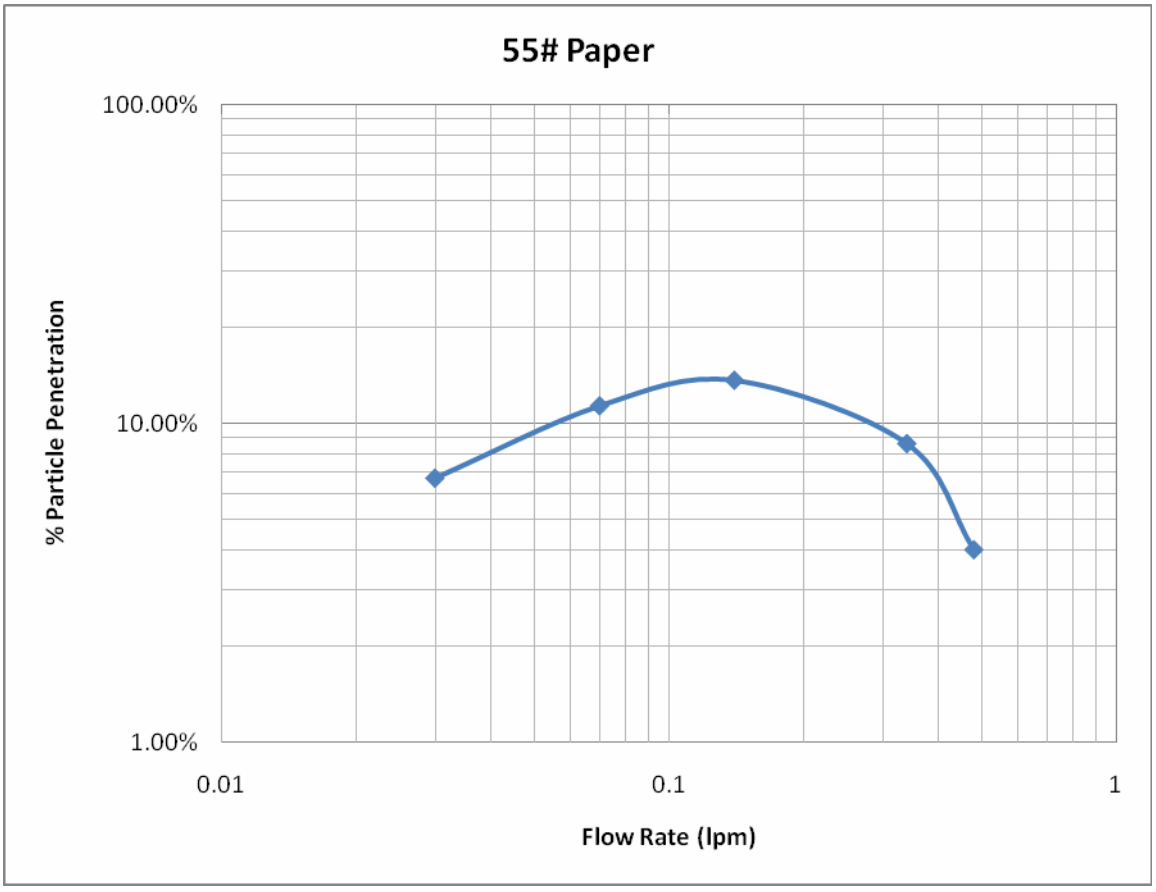
APPENDIX A: P_{max} Curves




55# Paper
16.00%
14.00%
12.00%
10.00%
8.00%
6.00%
4.00%
2.00%
0.00%
% Particle Penetration
0
0.1
0.2
0.3
0.4
0.5
0.6
Flow Rate (lpm)


Tyvek® 1073B
*also used for coated Tyvek® 1073B
10.00%
1.00%
0.10%
0.01%
% Particle Penetration
0.01
0.1
1
10
Flow Rate (lpm)


Ovantex
100%
10%
1%
% Particle Penetration
0.01
0.1
1
10
Flow Rate (lpm)


55# Paper
100.00%
10.00%
1.00%
% Particle Penetration
0.01
0.1
1
Flow Rate (lpm)

APPENDIX B: Data filtering technique

1. Raw Data- filtered for example test run time

Date	Time	Filtrate 0.7	Filtrate 1.0	Filtrate (0.7+1.0)	Challenge 0.7	Challenge 1.0	Challenge (0.7+1.0)	Sample dP (in H2O)	Vent Prsr (in H2O)	Vac Gen Prsr (psig)	Atomizer Prsr (psig)	Atomizer 1 Flow (lpm)	Atomizer 2 Flow (lpm)	Filtrate all	Challenge all	filtrate flow (lpm)
4/20/2009	11:03:00 AM	12	3	15	8326	1515	9841	0.065	0.873	1.8	31.2	1.36	2.02	238	42099	0.06
4/20/2009	11:03:06 AM	17	6	23	8305	1579	9884	0.065	0.866	1.8	31.2	1.33	2.02	273	42334	0.06
4/20/2009	11:03:12 AM	10	2	12	8408	1599	10007	0.065	0.864	1.8	31.2	1.33	2.02	272	41879	0.06
4/20/2009	11:03:18 AM	5	3	8	8470	1589	10059	0.065	0.863	1.8	31.2	1.33	2.02	249	42653	0.06
4/20/2009	11:03:24 AM	7	0	7	8415	1535	9950	0.065	0.862	1.8	31.2	1.33	2.02	276	42776	0.06
4/20/2009	11:03:30 AM	7	1	8	8482	1581	10063	0.065	0.863	1.8	31.2	1.33	2.02	257	42797	0.06
4/20/2009	11:03:36 AM	6	0	6	8597	1647	10244	0.065	0.862	1.8	31.2	1.33	2.02	216	42880	0.06
4/20/2009	11:03:42 AM	8	0	8	8640	1615	10255	0.065	0.862	1.8	31.2	1.33	2.02	253	43648	0.06
4/20/2009	11:03:48 AM	8	1	9	8758	1652	10410	0.065	0.862	1.8	31.2	1.33	2.02	243	43430	0.06
4/20/2009	11:03:54 AM	7	4	11	8558	1529	10087	0.065	0.863	1.8	31.2	1.33	2.02	233	42995	0.06
4/20/2009	11:04:00 AM	6	0	6	8748	1643	10391	0.065	0.863	1.8	31.2	1.33	2.02	230	42769	0.06

2. Data filtered for needed information- date, time, filtered particles 0.7 and 1.0 µm in diameter, challenge particles 0.7 and 1.0 µm in diameter, filtrate flow through sample.

Date	Time	Filtrate(0.7+1.0)	Challenge(0.7+1.0)	filtrate flow (lpm)
4/20/2009	11:03:00 AM	15	9841	0.06
4/20/2009	11:03:06 AM	23	9884	0.06
4/20/2009	11:03:12 AM	12	10007	0.06
4/20/2009	11:03:18 AM	8	10059	0.06
4/20/2009	11:03:24 AM	7	9950	0.06
4/20/2009	11:03:30 AM	8	10063	0.06
4/20/2009	11:03:36 AM	6	10244	0.06
4/20/2009	11:03:42 AM	8	10255	0.06
4/20/2009	11:03:48 AM	9	10410	0.06
4/20/2009	11:03:54 AM	11	10087	0.06
4/20/2009	11:04:00 AM	6	10391	0.06

3. A query was run (using MS Access) to link the appropriate time with the material sample and RH level tested.

Date	Time	Sample ID	Material ID	RH %	filtrate flow (lpm)	Challenge (0.7+1.0)	Filtrate (0.7+1.0)
4/20/2009	11:03:00 AM	B-90-6	B	90	0.06	9841	15
4/20/2009	11:03:06 AM	B-90-6	B	90	0.06	9884	23
4/20/2009	11:03:12 AM	B-90-6	B	90	0.06	10007	12
4/20/2009	11:03:18 AM	B-90-6	B	90	0.06	10059	8
4/20/2009	11:03:24 AM	B-90-6	B	90	0.06	9950	7
4/20/2009	11:03:30 AM	B-90-6	B	90	0.06	10063	8
4/20/2009	11:03:36 AM	B-90-6	B	90	0.06	10244	6
4/20/2009	11:03:42 AM	B-90-6	B	90	0.06	10255	8
4/20/2009	11:03:48 AM	B-90-6	B	90	0.06	10410	9
4/20/2009	11:03:54 AM	B-90-6	B	90	0.06	10087	11
4/20/2009	11:04:00 AM	B-90-6	B	90	0.06	10391	6

4. Penetration percentages were then calculated, Penrate=(challenge-filtrate/challenge).

Date	Time	Sample ID	Material ID	RH %	filtrate flow (lpm)	Challenge (0#7+1#0)	Filtrate (0#7+1#0)	penrate
4/20/2009	11:03:00 AM	B-90-6	B	90	0.06	9841	15	0.001524235
4/20/2009	11:03:06 AM	B-90-6	B	90	0.06	9884	23	0.002326993
4/20/2009	11:03:12 AM	B-90-6	B	90	0.06	10007	12	0.001199161
4/20/2009	11:03:18 AM	B-90-6	B	90	0.06	10059	8	0.000795308
4/20/2009	11:03:24 AM	B-90-6	B	90	0.06	9950	7	0.000703518
4/20/2009	11:03:30 AM	B-90-6	B	90	0.06	10063	8	0.000794992
4/20/2009	11:03:36 AM	B-90-6	B	90	0.06	10244	6	0.000585709
4/20/2009	11:03:42 AM	B-90-6	B	90	0.06	10255	8	0.000780107
4/20/2009	11:03:48 AM	B-90-6	B	90	0.06	10410	9	0.000864553
4/20/2009	11:03:54 AM	B-90-6	B	90	0.06	10087	11	0.001090513
4/20/2009	11:04:00 AM	B-90-6	B	90	0.06	10391	6	0.000577423

5. Averages were taken for the one minute time period for each sample (example shown for B-90-6 sample or 1073B with 90% RH at 0.06 lpm flow rate).

	Challenge	Filtrate	% Penetration
90	10452.91	5.333333	0.05%

REFERENCES

"Surgical and Medical Instruments and Apparatus." *Encyclopedia of American Industries*. Online Edition. Gale, 2009. Reproduced in Business and Company Resources Center. Farmington Hills, Mich.:Gale Group. 2009. http://galenet.galegroup.com/servlet/BCRC

"Surgical and Medical Equipment." *Encyclopedia of Global Industries*. Online Edition. Gale, 2009. Reproduced in Business and Company Resources Center. Farmington Hills, Mich.:Gale Group. 2009. http://galenet.galegroup.com/servlet/BCRC

American Society for Testing and Materials. (2007). *F2638-07: Standard Test Method for Using Aerosol Filtration for Measuring the Performance of Porous Packaging Materials as a Surrogate Microbial Barrier*. New York: American National Standards Institute.

American Society for Testing and Materials. (2006). *F4332-01: Standard Test Method for Conditioning Containers, Packages, or Packaging Components for Testing*. New York: American National Standards Institute.

American Society for Testing and Materials. (2004). *F1608-00: Standard Test Method for Microbial Ranking of Porous Packaging Materials (Exposure Chamber Method)*. New York: American National Standards Institute.

Bix, L., & De La Furnte, J. (2009). Medical Device Packaging. *Wiley encyclopedia of packaging technology: Third edition* (1-13).

Bowyer, J.L., Shmulsky, R, & Haygreen, J.G. (2003). *Forest products and wood science: An introduction.* Blackwell Publishing. Ames, Iowa. 447-448.

DuPont Company. (2007). *DuPont medical packaging: Technical reference guide*.

Dyro, J. F. (2004). Sterilization concepts for clinical engineers. *Clinical engineering handbook: Biomedical engineering*. Academic Press. 533-37.

Food and Drug Administration (FDA), Department of Health and Human Services. (2008). *Medical device classification procedures*. (21CFR860.3). Washington, DC: U.S. Government Printing Office.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards-a physics and chemistry*. Vol. 81A, No. 1, Jan-Feb.

International Standards Organization (ISO). (2006). *ISO 11607-1: Packaging for terminally sterilized medical devices-part 1: Requirements for materials, sterile barrier systems, and packaging systems*. Association for the Advancement of Medical Instrumentation.

International Standards Organization (ISO). (2006). *ISO 11607-2: Packaging for terminally sterilized medical devices-part 2: Validation requirements for forming, sealing, and assembly processes.*. Association for the Advancement of Medical Instrumentation.

Larget, B. *Randomized complete block design* [PDF document]. (2007). Retrieved from: http://www.stat.wisc.educourses/st572-larget/Spring2007/handouts15-4.pdf

Monty, C.P, and Mayers C.E, (1973). “Dust Contamination of Central Sterile Supply Packs.” *Nursing Mirror*. 137(6): 18-19.

Non-woven Fabric. (2009). In *Wikipedia online*. Retrieved May, 2009, from http://en.wikipedia.org/wiki/Nonwovens

Sherman, M. (Ed.). (1998). *Medical device packaging handbook*. New York: Marcel Dekker.

Sterilization (microbiology). (2009). In *Wikipedia online*. Retrieved May, 2009, from http://en.wikipedia.org/wiki/Sterilization_(microbiology)#Ethylene_Oxide

Tallentire A, and Sinclair C.S. (2002). Definition of a Correlation between Microbiological and Physical Particulate Barrier Performances for Porous Medical Packaging Materials. *PDA journal of pharmaceutical science and technology*. 56(1): 11-19.

Tallentire A, and Sinclair C.S. (1996). A Discriminating Method for Measuring the Microbial Barrier Performance of Medical Packaging Papers. *Medical Device and Diagnostics Industry*. 18(5): 228-241.

Tallentire A, and Sinclair C.S. (1986). The Influence of Dispersion Concentration on Microbiological Barrier Performance of Porous Packaging Materials. *Medical Device and Diagnostics Industry*. 8(8): 34-37.

Tallentire A, and Sinclair C.S. (1985). Microbiological Barrier Properties of Uncoated and Coated Spunbonded Polyolefin (Tyvek®). *Medical Device and Diagnostics Industry*. 7(10): 50-54.

Tallentire A, and Sinclair C.S. (1984). Permeability of Paper Webs to Air Flow-An Indicator of Structure. *Medical Device and Diagnostics Industry*. 6(5): 64-68.

Tallentire A, and Sinclair C.S. (1984). Variations in Structure and Microbial Penetrability of Uncoated Spunbonded Polyolefin (Tyvek®).” *Medical Device and Diagnostics Industry*. 6(7): 57-61.