

# STERILE FILTRATION VALIDATIONS

## NELSON LABORATORIES, INC.

Over the past thirteen years, Nelson Laboratories has established a solid reputation in the medical device and pharmaceutical industries for superior laboratory services. Filter Validation is just one of the areas where Nelson Labs is a manufacturer's first choice for laboratory services. Our laboratory is ISO 9001 certified and EN 45000 registered. We are routinely audited by FDA and other regulatory agencies. We welcome audit visits from client representatives and are anxious to provide our customers with all types of laboratory services and process recommendations.

Because of the growth in our filtration validation business, we have recently completed a new 200 square foot laboratory dedicated to Filtration Studies in our 25,000 square foot facility.

The filtration laboratory features HEPA filtered positive pressure (Class 1000) environment with filtered air, nitrogen and vacuum utilities and purified water (USP) plumbed in. We have validated in-line steam sterilization, ethylene oxide or steam autoclave facilities, and a complete supply of pumps, pressure vessels, filter housings and sanitary (type) plumbing fittings for simulating any process conditions.

Our laboratory includes self-contained sterility suites, BSL-3 facilities and numerous laminar flow HEPA filtered hoods and laboratories. Our on-site metrology laboratory ensures NIST traceable calibration accuracy for all measuring instruments.

We have completed filtration validation studies for many filter manufacturers and pharmaceutical companies.

## FILTRATION VALIDATION

The FDA defines<sup>1</sup> validation as:

"Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product, meeting its predetermined specifications and quality attributes."

In 1987, the U.S. Food and Drug Administration published<sup>2</sup> *A Guideline on Sterile Drug Products Produced by Aseptic Processing*. In this guideline, the FDA recommends that

whenever filtration is used as the aseptic step in the production process, validation procedures will include microbial retention testing of the filters. These tests need to be conducted using simulated pharmaceutical processing procedures which define a "worst case" production conditions. Parameters to be considered in these "worst case" conditions include: organism size, number of organisms, flow rate, temperature, pH, viscosity, pressure, etc.

The accepted industry standard<sup>3</sup> for "sterilizing grade" 0.2 $\mu$ m rated filters is the complete retention of *Brevundimonas* [the *Genus* name for this organism has been recently changed from *Pseudomonas*] *diminuta* ATCC #19146 at a concentration of at least  $1.0 \times 10^7$  organisms per square centimeter of effective filtration area at a differential pressure of 30 PSIG and a flow rate of at least 3.86 L/min/0.1 m<sup>2</sup>.

As the mechanism of retention in membrane filters is not only a sieving function, the chemical and physical properties of the product can have a significant influence on the effectiveness of the filter. It is therefore essential that the product's effect on the retention function is evaluated before traditional challenge testing. Filter compatibility studies may be required and the chemical and physical properties of the product need to be evaluated prior to design of the validation protocol.

The FDA Guideline<sup>2</sup> provides that products or product families which share similar attributes and processing conditions may be validated by a challenge procedure with that member which can be designated as "worst case."

Product families usually are consistent with one of three descriptions:

- ◆ Products containing different concentrations of identical ingredients. In this case, an acceptable validation would consist of the most dilute and the most concentrated sample.
- ◆ Identical products with different processing parameters. Here, it should be a straightforward choice of the "worst case" processing conditions (i.e., Batch size, flow rate, pressure, etc.)
- ◆ Those products with unique physical or chemical attributes which must be validated individually.

In each of these cases, small scale test conditions may be possible which will effectively simulate full-scale processing conditions for the product. It is essential that scaled down tests utilize the same filter membrane targeted for the actual process. There are, however, several concerns with scaled down tests. The test apparatus should simulate, as closely as possible, process conditions such as flow rate, pressure, contact time, and temperature. These parameters are interdependent, such that actual scale down may not be possible.

## **WORST CASE CONDITIONS**

The FDA<sup>2</sup> defines “Worst Case” as “a set of conditions encompassing upper and lower processing limits and circumstances, including those within Standard Operating Procedures, which pose the greatest chance of process or product failure when compared to ideal conditions.” Levy<sup>4</sup> *et. al.* , have discussed those effects of process and product on microbial retention of membrane filters which should be considered in the validation process. The effect of various process conditions on bacterial retention has been adequately demonstrated in the literature, but the mechanism and considerations for scale down in validations warrants some detailed discussion.

### **Filtration Pressure and Flow Rate**

The scientific literature confirms that pressure can have an effect on bacterial retention. Log reduction values (LRV), were shown to decrease as the pressure increased in research by Leahy<sup>5</sup>. The FDA’s “worst case” processing conditions definition would indicate a demonstration of bacterial retention at the highest expected differential pressure.

In the traditional test system, the interconnecting tubing diameter and attachment fittings are somewhat nonvariable so that in scaled down procedures, pressure and flow rate become interdependent. We have constructed our test apparatus with over sized tubing to eliminate as much as possible any flow rate limiting. In most validations, rate of flow per filter surface area is adjusted to match that of the actual process. We recommend validations at the highest flow rate on the assumption that these conditions will also result in the highest pressure differential. The recorded differential pressure may be higher or lower than the actual process pressure.

## **Hydraulic Process Conditions**

The hydraulic conditions of the process should be simulated during the challenge to assess what effect on the retentivity of the filter they might have. This includes intermittent or pulsed flow and changes in the differential pressure across the filter.

### **Temperature**

The temperature of the process may affect many of the physical parameters in the filtration process. Changes in viscosity may result in flow rate and pressure changes already discussed. Certain materials of construction in the filters may be adversely affected by elevated temperatures. From a bacterial challenge perspective, temperature can have a significant effect on the viability and recoverability of the microorganisms selected for the challenge. The challenge organism (i.e., *Brevundimonas diminuta*) has a relatively narrow temperature range for maintaining viability. Thermal shock to the challenge organisms could reduce the recovery process.

A re-circulating test can be used in cases where the process temperature does not permit the challenge organisms to survive. The drug product is recirculated through the filter at the elevated temperature for the prescribed duration to simulate temperature exposure and then the retention testing is conducted on the filter cooled down to a temperature which will preserve the organism’s viability.

### **Duration**

It has been demonstrated<sup>6</sup> that bacteria can migrate through filters in use for extended periods of time. While the mechanism for this migration is not clear, it is suggested that filtration processes do not exceed 24 hours in length. Validations for longer processes usually involve procedures for replenishment of the challenge and interim samples to ascertain bacterial breakthrough.

## **PRODUCT PROPERTIES**

The chemical and physical properties of the drug product can significantly affect the bacterial retention of filters used in the manufacturing process. Validation of the filtration process must address the possible interactions of each of these

properties. While each parameter may exert its specific effect on the retention process, the synergistic effect which is impossible to predict must be measured in the validation process. This is best accomplished by retention testing in the actual product.

### **Product Toxicity**

Of primary concern in the validation process is the effect of the product on the challenge organism. Many products are designed to inhibit bacterial growth or even have disinfectant properties. Other products intentionally incorporate antimicrobial ingredients. In some cases, bacterial toxicity may be a consequence of a synergistic activity of two or more ingredients, or a by product of various physical properties.

To a certain extent, product toxicity can be anticipated. Where no overt toxicity is presented by a review of the ingredients and physical properties, a toxicity test against the challenge organism should be performed. If the toxicity is two logs or less, the challenge titer can be adjusted up to compensate. If the kill is greater than two logs, the FDA Guideline<sup>2</sup> recommends substituting a nontoxic replacement for the toxic ingredient(s). It is essential, however, that the physical and chemical properties of the substitute are similar to the ingredient they replace.

If the active ingredient itself is toxic, or no acceptable surrogate ingredients can be found, the recirculating technique may be substituted for the actual product inoculation. In this case, the filter is operated in the product stream under process conditions and then the product is flushed out of the filter which is then challenged with the organism. This method exposes the filter to the product under “worst-case” conditions and then challenges the filter under conditions where the challenge organism can survive. These same considerations must be addressed for assay filters used in the validation testing as well.

Oil-based products, while not necessarily toxic, may interfere with the activity of the challenge organism. The FDA Guideline<sup>2</sup> indicates those challenges on oil-based products are not meaningful, and recommends substitution of the oil with other compounds of similar viscosity like glycerol. If this is not possible, the recirculating technique is suggested.

### **Surface Tension**

The presence of surface active ingredients may interfere with the bacterial retention mechanism of the filter, especially if the bacteria are smaller than the smallest pore in the filter. Recent research<sup>7</sup> has shown that sieving action may not be the primary retention mechanism in membrane filtration. Any phenomenon that might interfere with other filtration mechanisms must be considered in the validation process. The change in the “Bubble-Point” for the filter by the drug product does not, by itself, indicate a reduction in bacterial retention, but might be an indicator of the presence of surface-active ingredients.

### **Ionic Strength**

Hydrogen bonding between the challenge organism and the membrane may be one of the important retention mechanisms. As such, the physical property of the product which may interfere with this bonding activity must be addressed. Validations conducted in the actual product usually are sufficient to address these issues.

### **Osmolarity**

Small changes in the osmotic pressure between challenge microorganisms and the solution can significantly alter the size of the bacterial cell<sup>8</sup>. As water leaves the high concentration within the cell to a dilute carrier fluid, cell shrinkage can occur which may negatively bias the validation. Conversely, water forced into the challenge bacteria from a highly concentrated carrier may cause the cells to swell artificially enhancing their filterability.

### **pH**

In addition to the effect pH may have on the viability of the challenge organism, it can significantly affect the charge mechanism thought to be a primary retention mechanism for bacteria in membrane filtration. Like surface tension and osmolarity, pH may interfere with the effectiveness of membrane filters that retain particles by adsorption.

## Viscosity

Products of high viscosity must usually be processed at elevated temperatures if they are to be filtered. A validation performed at lower temperatures slows the flow rate which conceivably could enhance the retention properties of filters operating under adsorptive mechanisms. Maintaining the elevated temperature (i.e.,  $>40^{\circ}\text{C}$  would be deleterious to the challenge organism.) Replacement of the viscous component or the recirculating technique may be called for in validating filtration for highly viscous materials.

Under the FDA Guideline, manufacturers can validate sterile processing if the process produces a sterile effluent when challenged with *Brevundimonas diminuta* at a density of at least  $1.0 \times 10^7$  organisms per square centimeter of filtration surface. If the recirculation technique is required, the validation report must contain a statement concerning the toxicity of the product, or the inappropriateness of a particular physical parameter.

At first glance, it would appear that a matrix could be constructed from historical data which would indicate the range of acceptability for each parameter and product type. Then, if the product description fell within this matrix, one might conclude that the filtration process was validated. This matrix approach has been employed in some validations, but in many cases, the FDA has rejected data from this method when the synergistic effect of more than one parameter has not been adequately addressed. In most cases, a more acceptable approach documents actual challenge data on actual products. This ensures compliance with even the most stringent regulatory requirements.

## PROTOCOL

In response to requests by pharmaceutical manufacturers for assistance in validating their process, Nelson Laboratories supplies a Pre-Feasibility Study Information form which the manufacturer completes. This form conveys to the laboratory as much information about the product and process such that a detailed protocol can be written.

## Feasibility Studies

Several preliminary tests are necessary to define what effects the

product may have on the viability and dispersion of the challenge organisms. To prove that the challenge organism can remain viable and monodispersed in the product over the duration of the test, the product is inoculated with the challenge organism. The suspension is incubated at process temperature and samples are removed and assayed at appropriate intervals. If viability is degraded or cell aggregation is observed, a determination must be made about the appropriateness of the inoculated product approach or if the recirculating technique must be used. If bacteriocidal or bacteriostatic activity is observed, a residual effect test must be performed before the recirculating technique is used. In the residual effect test, a volume of the product is filtered and then rinsed from the membrane with an appropriate solvent. A small quantity of sterile deionized water is then filtered and inoculated with a dilute challenge. Assays of the filtrate compared to a control without the product are compared to verify the adequacy of the filter rinse procedure. A separate protocol may be required to determine if there are any interferences in the product or process which might affect the validation study. Using the information obtained in the feasibility study, Nelson Laboratories develops a validation protocol for the product which incorporates the FDA Guidelines and industry practice for the product and process. When the protocol is accepted by our client, we schedule the microbial testing.

This protocol describes the test procedure for evaluating bacterial retention characteristics of membrane filters used in the sterilizing process. At least three filters from different manufacturing lots are tested in the validation. Each test filter is challenged with a suspension (usually in the actual product matrix) of *Brevundimonas diminuta* ATCC #19146 that has been specially cultured to maximize the percentage of organisms capable of passing through a typical  $0.45 \mu\text{m}$  filter. Sufficient volume of this challenge suspension, equivalent to at least  $1 \times 10^7$  CFU per  $\text{cm}^2$  of effective filtration area (EFA) will be prepared for each filter. The challenge is conducted at a high flow rate and at a maximum differential pressure consistent with "worst case" processing conditions. The effluent is collected and assayed quantitatively by membrane filtration. Integrity testing<sup>9</sup> is performed before and after the bacterial challenge procedure.

The selection of *Brevundimonas diminuta* as the challenge organism is based on literature reports<sup>10</sup> that the organism attains a very small size when grown under stress or minimal nutritional conditions. The test procedure complies in intent and content with the ASTM F838-83 Standard Test Method<sup>11</sup>

"Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration" and the Health Industry Manufacturers Association (HIMA) Test Method<sup>12</sup> "Microbiological Evaluation of Filters for Sterilizing Liquids". Saline Lactose Broth is selected as the growth media because the literature reports that this media in concert with carefully controlled growth conditions, results in a minimally aggregated population of *Brevundimonas diminuta* cells, a significant percentage of which, will pass through 0.45  $\mu\text{m}$  membranes. The test objective of a most severe bacterial challenge to the filter is met by the challenge conditions which include high pressure, high flow rates and a high bacterial concentration per  $\text{cm}^2$  of effective filtration area. The growth parameters, temperatures and media are adapted from the ASTM and HIMA methods. The test filters must completely retain the challenge for the process to be validated.

### **Inoculated Product Challenge**

When no deleterious effects from the product are revealed in the feasibility study, the organism is suspended directly in the product to a concentration resulting in greater than  $1.0 \times 10^7$  organisms per square centimeter of effective filtration area. A sample of the challenge suspension is taken for titer and the test filters are challenged at "worst-case" conditions. The filtrate is collected and the total volume assayed by membrane filtration to quantitate any passage of the challenge organism through the test filter. Integrity testing (Forward air diffusion and bubble point) is performed on each filter before and after the challenge.

### **Recirculating Technique**

When toxicity or physical incompatibilities direct, the product is recirculated through the test filters by peristaltic pump for the duration of the process time. The filter and test apparatus is flushed with an appropriate solvent to remove any residual product and the filter is challenged with the organism at  $1.0 \times 10^7$  per  $\text{cm}^2$  in Saline Lactose Broth. The filtrate is collected and assayed to quantitate any passage of the challenge organism through the test filter. Integrity testing (Forward air diffusion and bubble point) is performed on each filter before and after the challenge.

### **Positive Control**

The ability of a significant number of organisms from the test challenge suspension to pass through a 0.45 $\mu\text{m}$  membrane constitutes the positive control. It is verified concurrent with challenges by challenging a "Standard" cellulose ester 0.45 $\mu\text{m}$  membrane with an aliquot of the challenge suspension. The log reduction value (LRV) for the "Standard" must fall within two standard deviations when compared with the historical data for the control.

### **Laboratory Quality Assurance Procedures**

Filtration validation studies for pharmaceutical products are most often carried out under GLP conditions which include documentation and quality assurance procedures designed for acceptability by the U.S. FDA. Even when not specifically identified as a GLP study, all studies at Nelson Laboratories include the following:

- ◆ All general laboratory procedures are conducted in accordance with published Standard Operating Procedures (SOPs)
- ◆ All pressure gauges, flow meters and timers are calibrated periodically against standards traceable to NIST.
- ◆ All steam sterilizers are validated.
- ◆ Temperatures of incubators are monitored and recorded.
- ◆ The growth promoting ability of media used in assay plates is verified.
- ◆ All measuring equipment is calibrated and maintained as per written company SOPs and schedules (i.e., balances, pH meters, etc.).

### **Terms and Conditions of Validation Studies**

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