In this issue we present two articles on the topic of “sterilization”.

The first article is by Dr. Tim Sandle. For those who have experienced a biological indicator failure, you know that determining the root cause of the failure is a daunting task, often resulting in no definitive root cause. Dr. Sandle presents a pragmatic and thorough process for investigating BI failures. He categories each failure type, and provides corresponding detailed investigations points.

The second article was co-authored by myself, Mark Fosbenner and Eric Staib. Often, companies assign hold times (e.g., 30 days) for sterilized materials and equipment without a scientific rationale for the hold time. In particular, an “event-based” hold time is typically not considered. We present a regulatory and historical perspective on the topic, and describe the process for validating a hold time.

We hope you enjoy this issue of the PMF Newsletter. As always, feel free to contact us if you have any questions or comments, or wish to author an article for a future issue.

Bob Westney rwestney@cryologics.com
Introduction

Biological indicators are an established part of the qualification of steam sterilizers. Biological indicators are used, in addition to use of thermocouples for physical measurements, to determine the effectiveness of sterilization cycles (the common sterilization methods being gamma irradiation, gas sterilization, dry heat and moist heat). Steam sterilization devices, such as autoclaves, are the most common sterilization devices in pharmaceutical processing. Such devices go through initial qualification and re-qualification (either six-monthly or annually). Both qualification phases require the use of biological indicators.

Biological indicators are preparations of bacteria spores of a defined population, purity and resistance on a carrier ready to use and providing defined resistance to the specified sterilization process. The carrier is a solid support upon which the test microorganism is inoculated. Biological indicators are used to measure the effectiveness of a sterilization system to destroy or reduce the spore population. The reduction in population is measured in terms of logarithmic reduction (log10).

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Biological indicators are primarily used to measure the effectiveness of sterilization (refer to USP <1211> Sterilization and Sterility Assurance of Compendial Articles). Biological indicators need to be of a certain microorganism, population and D-value appropriate to the sterilization process.

The D-value (or D10 value) is the time in minutes required for a one logarithm or 90% reduction of the population of the microorganisms used as a biological indicator under specified lethal conditions (PDA Technical Report No. 51). The population used is >5.0 x 10^5. This meets the PDA recommendation that the micro-organism used as the biological indicator should have a higher population and resistance than the bioburden on the item to be decontaminated (PDA Technical Report No. 51). The biological indicators used can be readily enumerated.

The micro-organism used must be appropriate, that is having a resistance to the sterilization process (such as the use of Bacillus atrophaeus spores to monitor ethylene oxide sterilization and dry heat sterilizers). For steam sterilization this is normally Geobacillus stearothermophilus. The micro-organism used must be traceable to an approved culture collection (such as the American Type Culture Collection: ATCC 7953). The presentation of the biological indicator is often as bacterial endospores on a paper carrier in a glassine envelope. After use within the steam sterilization device, the paper carrier is placed within soya-bean casein digest medium and incubated at 55-60°C for at least seven days.

Although most steam sterilization devices and sterilization cycles are well designed, there are a number of occasions where biological indicators can fail. A positive test result from a biological indicator can result from a variety of causes, such as inadequate steam quality, insufficient exposure time or temperature, poor loading practices, or product failure or operator failure. This article considers some of the scenarios (Continued on page 3)
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which can cause a biological indicator to fail and some of the areas to investigate.

Scenarios causing biological indicator failure

There are several scenarios which can lead to a biological indicator failure, which are discussed below. The key to any investigation is the collection of information and this remains so with biological indicators and steam sterilization devices. As a minimum the following information should be noted as standard for each run:

• The lot/load number of the sterilization run.
• The contents of the sterilization load.
• The exposure time and temperature of the sterilization run.
• That the temperature and pressure of the chamber during a sterilization cycle were within specifications.
• If there was any leakage of steam, water or effluent at any point during the sterilization cycle.
• The name of the operator (which should prompt a review of the operator's training record).
• The results of the biological indicator test.
• The response of the chemical indicator placed in the biological-indicator test packs.
• The results of leak testing (checking that the leakage into the chamber during a vacuum cycle does not exceed the specified maximum).
• Any reports of inconclusive or non-responsive chemical indicators found later in the load

Where a failure occurs, the following information should also be noted:

• The time and date of the questionable sterilizer cycle.
• A description of the sterilizer and load, with reference to the appropriate load control number.
• The results of mechanical monitoring and of the internal chemical indicator test (if applicable) as obtained from the user department.

Having detailed records allows operational issues, laboratory issues and preparation issues to be examined (as discussed below).

A. Operational issues

A number of operational issues can arise with the steam sterilization device which might cause, for example, ‘cold spots’ to develop. Such issues include:

• Shape/size/volume of containers and materials
• Thermoconductive properties of containers and contents
• Density of solid materials and viscosity of liquids
• Position of load within the chamber
• Load configuration

Operational issues and some possible causes are examined in Table 1 (page 4).
The primary causes for biological indicator failure most likely relate to issues with steam quality or temperature. This can arise because of:

- Wet steam: where there is an inadequate trap in steam line, steam contact with a cold load, or where the steam pressure too high for the temperature.
- Superheated steam: this can relate to improper chamber heat up, desiccated packaging materials, or where the steam pressure too low for the temperature.
- Variations in steam pressure: such as being due to clogged filters, poorly engineered piping or excessive demands.
- Out-of-calibration pressure gauges and controllers: These can lead to incomplete air removal, plugged drain screen, clogged vent lines, faulty vacuum pump, inadequate door gasket seal, low steam pressure, plugged, faulty or maladjusted control valves, or come up time less than 1.5 minutes.
- Inadequate cycle temperature: temperature gauge out of calibration, long heat-up time of large loads (heat lag), variations in steam pressure due to clogged filters, poorly engineered piping, poorly engineered chamber.

(Continued on page 7)

Table 1

<table>
<thead>
<tr>
<th>Failure</th>
<th>Investigation to look at Acceptable Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient Time/ Temperature Conditions</td>
<td>Sterilization cycles (such as 121°C and 134°C cycles) need to be established. Has all thermometric data been confirmed to meet the required acceptance criteria?</td>
</tr>
<tr>
<td>Air in the Chamber due to Non Condensable Gases (NCG)</td>
<td>Verify NCG, Dryness and Superheat values. Typical values are: NCG&lt; 2.5%, Superheat &lt; 25°C and Dryness &gt;0.95.</td>
</tr>
<tr>
<td>Change in Load Configuration (unintended changes to load)</td>
<td>No physical values can be assigned. It is important to train staff in identification against a critical load, especially prior to placing thermocouples and BIs.</td>
</tr>
<tr>
<td>Change in orientation and presentation of load</td>
<td></td>
</tr>
</tbody>
</table>

Comment: Whilst Production personnel are trained in load preparation, it may not be that validation personnel have the same level of awareness.
INTRODUCTION

In general, the length of time an item is or can be considered sterile depends on many factors. These factors include the type and configuration of packaging materials used or applied, the number of times a package is handled before use, the number of personnel who may have handled the package, storage location of the equipment or item such as an open shelf, closed cabinet or unclassified warehouse, the environmental conditions of the storage area (e.g., cleanliness, temperature, humidity), and the use of dust covers and method(s) of sealing. No matter what the item, or the size of the equipment, all of these factors and more contribute dramatically to the “time limit” / “hold time” of sterilized objects. The difficulty this presents however, is the control, validation, and monitoring of these factors within the manufacturing of a drug product or biologic. This defined “time/period” must be experimentally determined, but how and in what way to make it feasible?

Regulatory Requirements & Guidance

Specific regulatory requirements related to sterility and the prevention of contamination of containers & closures, etc. can be found under 21 CFR Part 211.80(a), 211.80(b), and 211.111.

- 21 CFR 211.80(a) states that, “There shall be written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of components and drug product containers and closures; such written procedures shall be followed.”

- 21 CFR 211.80(b) states that, “Components and drug product containers and closures shall at all times be handled and stored in a manner to prevent contamination.”

- 21 CFR 211.111 states that, “When appropriate, time limits for the completion of each phase of production shall be established to assure the quality of the drug product. Deviation from established time limits may be acceptable if such deviation does not compromise the quality of the drug product. Such deviation shall be justified and documented.”

Additionally, and more specifically, are those recommendations found within the September 2004 Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing – cGMP. The guidance contains two very specific directives that pertain to the topic.

1. Section VI (B). “Written procedures should specify the frequency of revalidation of these processes as well as time limits for holding sterile, depyrogenated containers and closures.”

(Continued on page 6)
2. Section VIII. “Time limits should include, for example, the period between the start of bulk product compounding and its sterilization, filtration processes, product exposure while on the processing line, and storage of sterilized equipment, containers and closures.”

These two guidance document statements are basically a further clarification of 21 CFR 211.80(a) and (b) respectively, and include time limits as mentioned in 21 CFR 211.111. A practical application of this FDA expectation(s) follows:

During an inspection of Bioport Corporation (Lansing, MI) from November 11th to the 15th, 1999, the FDA issued a 483 observation for the absence of the firm to evaluate the integrity of product containers and closures, and determine “sterility hold times” for Alhydrogel, bulk formulation tanks, fermentors, harvest tanks, glassware, etc. The specific reference was that the ability of the items to remain sterile was never incorporated into the validation of the process or processes to sterilize these items. This particular organization was also found (i.e. in terms of aseptic operations) to not be “incubation testing” closures such as an entire formulation bottle or even the media prepared within various manufacturing vessels used in order to produce their vaccine.

DISCUSSION

Categorizing equipment and component configurations, within the context of the method of their sterilization, establishes the initial approach to qualifying their hold time. For example, SIP (Steam- or Sterilization-In-Place) of a large-scale compounding tank warrants an evaluation different than autoclaving forceps.

Equipment sterilization has a relatively well-accepted methodology as to how to develop and challenge sterilization processes. Currently, advanced aseptic processing technologies support effective and controlled sterilization of large-scale manufacturing equipment such as tanks, piping, connections, manifolds, etc. Subsequent to their sterilization, these types of equipment are usually held in the same cleanroom in which they were sterilized, or transported in well-controlled (classified) cleanroom environments. These equipment are typically integral and of a “closed-system” design, effectively precluding incidental microbial contamination after sterilization.

Qualifying the hold time, or expiration, of smaller equipment and components presents a more challenging approach. Sterilization methods include: steam (e.g., autoclaving), heat (e.g., depyrogenation), radiation (e.g., gamma radiation), and gas (e.g., ethylene oxide [ETO], vaporized hydrogen peroxide [VHP]). The type of barrier used to pro-

(Continued from page 5)
piping or excessive demands on the steam supply.
- Insufficient time at temperature: timer gauge out of calibration, inappropriate cycle parameters for the load being processed, or. Again, come up time less than 1.5 minutes.
- Human error: This is a wide ranging area. In this context this can refer to inadequately cleaned items preventing steam penetration, packaging materials impermeable to steam, packs too large or too dense for the cycle parameters, poor loading techniques that entrap air and prevent steam penetration, incorrect operation of sterilizer entire load inadvertently not processed.

Thus the key cycle parameters needed for steam sterilization are time, temperature and saturated steam.

B. Laboratory issues
Various laboratory issues can arise with the receipt and testing of biological indicators which can lead to errors arising. Furthermore, variations can arise between biological indicators (the indicator is not, like other biological reagents, easy to ‘standardize’). These issues are captured in Table 2 (page 8).

C. Preparation of steam sterilization device
The third set of scenarios relate to the preparation of the steam sterilization device. These are presented in Table 3 (page 9).

Investigations into biological indicator failures
The investigation into a biological indicator failure should include the Microbiology department. There are a number of variables to consider. Table 4 (page 10) details some of the areas for investigation.

Summary
This paper has examined the use of biological indicators for steam sterilization studies and considered some of the scenarios which might cause a biological indicator to fail and some of the areas to examine when investigating a biological indicator failure. As with all areas of microbiological testing, a robust investigation with appropriate corrective and preventative actions is essential.

References
- Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 4th edition. April 1999
- National Standards and Recommended Practices for Sterilization. Association for the Advance-
Investigation to look at Acceptable Parameters

<table>
<thead>
<tr>
<th>Failure</th>
<th>Investigation to look at Acceptable Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>High D-Value BI Variability</td>
<td>Confirm the D value and population. Data should be collected to indicate what the variability on certificated population and D values is? [I have seen values in literature from 50% &lt; X &lt; 200% (or 300%) for population and ±20% for D value]</td>
</tr>
<tr>
<td>Lab Error</td>
<td>Preparations of BIs: The risk is mislabelling. This should be covered by a procedure and the risk is low.</td>
</tr>
<tr>
<td></td>
<td>Loading: The risk is cross contamination. There should be a procedure in place to minimize the risk.</td>
</tr>
<tr>
<td></td>
<td>Receipt of BIs: There is a risk of losing a BI. There should be a primary check by the engineer removing the BI’s and a secondary check in the Microbiology laboratory.</td>
</tr>
<tr>
<td></td>
<td>Controls: Each test run should have a positive control (to show that BI’s used were viable and of the correct strain) and a negative control (to show that the culture media was not contaminated)</td>
</tr>
<tr>
<td></td>
<td>Misread of BIs: Staff reading BI’s should undergo an annual test to spot turbid vials, a misread is unlikely.</td>
</tr>
<tr>
<td>Cross-contamination post sterilization</td>
<td>Separation of Positive Control and sterilized BIs should ensure there is none of this. Good aseptic technique is required.</td>
</tr>
</tbody>
</table>

(Continued from page 7)

- PDA Technical Report No. 51 ‘Biological Indicators for Gas and Vapor-Phase Decontamination Processes: Specification, Manufacture, Control and Use’

About the Author:

Dr. Tim Sandle is the Head of Microbiology at Bio Products Laboratory Limited. In addition, Dr. Sandle is an honorary consultant with the School of Pharmacy and Pharmaceutical Sciences, University of Manchester and is a tutor for the university’s pharmaceutical microbiology MS course. Dr. Sandle serves on several committees relating to pharmaceutical microbiology and cleanroom contamination control including BSI cleanroom standards and the UK Pharmaceutical Microbiology Interest Group. Dr. Sandle has written over one hundred papers, book chapters and technical articles and he also runs an on-line microbiology blog (www.pharmig.blogspot.com).
## Table 3

<table>
<thead>
<tr>
<th>Failure</th>
<th>Investigation to look at Acceptable Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-contamination post sterilization</td>
<td>Separation of Positive Control and sterilized BIs should ensure there is none of this. Good aseptic technique is required.</td>
</tr>
<tr>
<td>Positioning of BIs within the load</td>
<td>Prior to and post – run: Do any of the test sheet comments identify if any BIs moved or were damp/wet after removal?</td>
</tr>
<tr>
<td>Accidental errors in presentation of load (i.e. kinked tubing, valve closed)</td>
<td>Investigation needs to confirm if there were any issues noted at the time of execution.</td>
</tr>
<tr>
<td>Wet/Damp BI spore strips on removal</td>
<td>Dry is perfect. Wet is bad and may have reduced the effectiveness of the cycle. Documentation should identify if any BIs were wet.</td>
</tr>
<tr>
<td>Dislodged spore strips (and thermocouple) after the cycle has completed</td>
<td>The strips and thermocouples are required to remain in place during the cycle.</td>
</tr>
<tr>
<td>Autoclave functionality failure.</td>
<td>Failed Air Detector Performance Test or Autoclave Pack Test</td>
</tr>
<tr>
<td>Undocumented change (unauthorised modifications) of critical components (i.e. paper wrapping or autoclave bag)</td>
<td>Investigate and identify any change in materials. Lot track all critical components of load and identify if the lot no of the item changed.</td>
</tr>
<tr>
<td>Incorrect calibration of in-situ instrumentation</td>
<td>All instruments are subject to strict calibration procedures. The only pathway for such a scenario would normally be for multi-instrumental failure, which should be a very rare event.</td>
</tr>
<tr>
<td>Incorrect calibration of Kaye Thermocouples/Pressure Transducer</td>
<td>Thermocouples and pressure transducers are calibrated immediately before and then immediately after any autoclave work.</td>
</tr>
<tr>
<td>Change in specification of in-situ instruments</td>
<td>This should fall under appropriate change controls.</td>
</tr>
<tr>
<td>Steam usage and consistency of supply to header</td>
<td>Investigation can identify if the physical values have been met: Clean Steam Generator 4.0 -5.0 barg Clean Steam Reduced to 3.0 to 3.5 barg DEMIN feed to CSG &lt;5μS/cm (&lt;4.3 shut-off) Clean Steam Condensate &lt;1μS/cm</td>
</tr>
</tbody>
</table>
Table 4. Possible Cause / Area to Investigate

<table>
<thead>
<tr>
<th>Main Area</th>
<th>Investigate:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sampling</strong></td>
<td>Aseptic technique</td>
</tr>
<tr>
<td></td>
<td>Correct biological indicator used (released; D-value; population; time expiry)?</td>
</tr>
<tr>
<td></td>
<td>Adventitious contamination</td>
</tr>
<tr>
<td></td>
<td>Consumable/reagents/media – satisfactory?</td>
</tr>
<tr>
<td></td>
<td>Identify who took the sample</td>
</tr>
<tr>
<td></td>
<td>Staff Training</td>
</tr>
<tr>
<td></td>
<td>Integrity of containers</td>
</tr>
<tr>
<td></td>
<td>Correct number of samples sent over?</td>
</tr>
<tr>
<td></td>
<td>Placed in correct locations?</td>
</tr>
<tr>
<td></td>
<td>Examination of results from previous tests</td>
</tr>
<tr>
<td></td>
<td>Transportation</td>
</tr>
<tr>
<td></td>
<td>Storage (pre and post-use). Storage can affect the spore resistance</td>
</tr>
<tr>
<td><strong>Test Method</strong></td>
<td>Storage</td>
</tr>
<tr>
<td></td>
<td>Time expiry of biological indicators after removal from sterilization device?</td>
</tr>
<tr>
<td></td>
<td>Reconciliation of returned BIs</td>
</tr>
<tr>
<td></td>
<td>BI envelope wet?</td>
</tr>
<tr>
<td></td>
<td>BI envelope intact?</td>
</tr>
<tr>
<td></td>
<td>Interpretation of result / calculations</td>
</tr>
<tr>
<td></td>
<td>Aseptic technique satisfactory?</td>
</tr>
<tr>
<td></td>
<td>Calibration of equipment</td>
</tr>
<tr>
<td></td>
<td>Training of tester satisfactory?</td>
</tr>
<tr>
<td></td>
<td>Media satisfactory / expiry time?</td>
</tr>
<tr>
<td></td>
<td>Test incubation correct? / incubation parameters correct?</td>
</tr>
<tr>
<td></td>
<td>Purity?</td>
</tr>
<tr>
<td></td>
<td>Satisfactory identifications?</td>
</tr>
<tr>
<td></td>
<td>Satisfactory controls?</td>
</tr>
<tr>
<td></td>
<td>Consumables – integrity / expiry date</td>
</tr>
</tbody>
</table>

(Continued on page 11)
**Table 4. Possible Cause / Area to investigate (cont’d.)**

<table>
<thead>
<tr>
<th>Main Area</th>
<th>Investigate:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A failure to demonstrate kill, after appropriate investigation, may suggest that one or more operational parameters has drifted. This would result in a review of all testing and necessary repairs being made.</td>
</tr>
<tr>
<td></td>
<td>Result of unacceptable variation in the resistance of commercially available biological indicators: this can occur due to multi-layers of spores that increase the overall resistance of the biological indicator. This can result in unexpected late growth occurring in biological indicators that have been subject to well-established sterilization cycles.</td>
</tr>
<tr>
<td></td>
<td>Check sterilization cycle parameters</td>
</tr>
<tr>
<td></td>
<td>Review test protocol / SOPs</td>
</tr>
<tr>
<td></td>
<td>Review equipment calibration data</td>
</tr>
<tr>
<td></td>
<td>Review maintenance records</td>
</tr>
<tr>
<td></td>
<td>Review trends from previous cycles</td>
</tr>
<tr>
<td></td>
<td>Review temperature data (and chemical indicators if appropriate)</td>
</tr>
<tr>
<td></td>
<td>Consider re-running the failed cycle with a different lot of biological indicators</td>
</tr>
<tr>
<td></td>
<td>Consider the use of duplicate or triplicate biological indicators in the suspect location</td>
</tr>
<tr>
<td>Other factors</td>
<td>Other factors to investigate may include temperature, dew point time and the extent to which surfaces are clean and dry.</td>
</tr>
<tr>
<td></td>
<td>Some materials used to form the primary pack may have poor gas permeability which can result in increased spore inactivation times</td>
</tr>
<tr>
<td></td>
<td>Was the carrier used suitable?</td>
</tr>
<tr>
<td></td>
<td>When checking confirmatory results for D-value tests by a contract laboratory it is noted that the D-value will vary according to the resistometer (such as a BIER vessel) used. Further variations can occur with different culture media and the method used to interpret the results.</td>
</tr>
<tr>
<td></td>
<td>Factors which can affect a biological indicator failure include: variation of the inoculation of the carrier; genotypic variation amongst spore population; aggregation of spores; presence of cell media / debris; orientation of the carrier within the primary package; surface characteristics of the carrier; handling of naked biological indicators (outside of the carrier) by operators; method to release spores from carrier; age of biological indicators used; retention of sterilant in carrier; time between exposure and recovery of biological indicators; composition of recovery medium; pH of recovery medium.</td>
</tr>
</tbody>
</table>
tect the item from incidental microbial contamination subsequent to sterilization must be a function of the sterilization method. For example, a paper or plastic wrap is inappropriate for heat sterilization. The size, shape and materials of construction must be considered in order to establish the most appropriate barrier. For example, for steam sterilization, a paper barrier may be inappropriate for a large, heavy stainless steel object with sharp edges, while it may be appropriate for small, lightweight rubber objects such as stoppers. Transport and storage of sterilized items may also vary according to facility layout (e.g., adjoining classified areas), manufacturing processes, storage capacity, etc.

Given the variety of circumstances associated

with sterilization, transport and storage of these types of equipment and components, little industry, compendial or regulatory guidance currently exists for qualification of sterility expiry. Studies of the expiry of sterilized packaged materials have been performed for many years. In one of these studies\(^1\), the integrity of various wrapping materials (two-ply reusable, nonbarrier wovens; disposable, barrier nonwovens; and polypropylene peel pouches) was evaluated under different storage times and conditions. The authors concluded, “no trend toward increased probability of contamination over time was observed for any of the pack types studied.” Another study evaluated the sterility of implant components in their original glass vial and peel-back packages beyond their expiration date\(^2\). A military study conducted at Wilford Hall USAF Medical Center\(^3\) investigated the potential shelf life of three packaging materials for sterile instruments, and the investigators concluded that “the results of the studies indicate that sterility is maintained for at least 1 year” for the three materials evaluated.

The limitation of these types of studies, beyond the variations in storage conditions, was the exclusion of handling of the packaged sterilized items. Several studies have been conducted to evaluate event-related versus time-related challenges to package sterility. In 1981, Philips reported that “maintenance of sterility of commercially sterilized medical devices is event related, not time related”\(^4\). According to the Association of periOperative Registered Nurses (AORN) "Recommended practices for sterilization in perioperative practice settings," shelf life of a packaged sterile item is event related. AORN maintains that the length of time an item is considered sterile depends on (a) the type and configuration of packaging materials used, (b) the number of times a package is handled before use, (c) the number of personnel who may have handled the package, (d) storage on open or closed shelves, (e) the environmental conditions of the storage area (e.g., cleanliness, temperature, humidity), and (f) the use of dust covers and method of seal-

(Continued from page 6)
ing. The University of Rochester Medical Center provides a succinct approach to justifying event related sterility expiry of hospital-prepared packages containing sterile supplies wrapped in single-use or reusable materials. Implementation of this approach includes not only defining “events that would require reprocessing of packages”, but also a “review of technical documentation on barrier quality of packaging materials.”

Some guidance can be gleaned from compendial and regulatory publications. The scope of USP 34 <1207> pertains to container-closure systems of drug products and to sterile barrier packaging of medical devices, and provides this guidance: “Product package integrity testing continues throughout the life cycle of the product. Generally, this integrity testing should occur during three phases: (1) the initial development of the product packaging system, (2) routine manufacturing, and (3) shelf life stability assessments.” For selection of evaluation methods, this informational chapter recommends that “physical and microbiological methods for product package integrity testing should take into consideration [a] the design of the closure system; [b] the manufacturing method, including the sterilization process; and [c] the intended use of the product.” Therefore, an inference can be made to [a] the design of the packaging used for items to be sterilized, [b] the method of sterilization, and [c] the storage of the sterilized item. Additional compendial guidance can be inferred from USP 34 <1079>, which describes the approaches to evaluating the temperature, humidity and physical challenges of packaging components within the context of storage, distribution and shipment of final drug product. The Food and Drug Administration published a guidance document in 1994, “Guidance for Industry for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products”, which addresses container-closure and package integrity. The Guidance recommended that the following types of information be addressed in integrity studies: (1) simulation of the stresses from processing, (2) demonstration of integrity following the maximum [sterilization] exposure, (3) multiple barriers, (4) sensitivity of the test [method], and (5) integrity over the product shelf life.

The basic approach to establishing baseline conditions for qualifying or evaluating the shelf life (or expiry) of sterilized materials should include: (A) method of sterilization, (B) evaluation of the material used to wrap or protect a given item, (C) method of wrapping/protecting the item, and (D) transport/storage conditions. As discussed above, some guidance can be derived from industry, compendial or regulatory publications. In addition, in a series of nine “recommended practices”, AORN offers a highly relevant and detailed approach to “evaluation, selection, and use of packaging systems for items to be sterilized.” This approach holds merit for the pharmaceutical and biotechnology industries, since specific guidance is provided
for several scenarios that can be applied to cGMP manufacturing processes and environments. The approach discussed below incorporates many aspects of these “recommended practices”. By establishing a systematic, standardized approach to sterilizing and storing materials and equipment, qualification of the hold time (i.e., expiry) of these materials and equipment can be achieved.

Method of Sterilization
Sterilization methods are well characterized, including the physical and/or chemical modes of action. Extreme physical conditions, such as temperature and pressure, should be considered to determine the method that is least detrimental to not only the item’s materials of construction but also the material of the wrap/package barrier.

Evaluation of Material
Materials should be compatible with and able to withstand the physical conditions of the sterilization process. Packaging systems for all sterilization methods should permit use of material compatible (i.e., nondegradable) with the sterilization process and maintain the integrity of the system. Packaging systems for steam sterilization should provide adequate air removal, permit steam penetration and direct contact with item(s) surfaces, and permit adequate drying. Packaging systems for ETO should be permeable to ETO, moisture, and air, and permit aeration. Packaging systems for VHP sterilization should be permeable to the process, be made of a material recommended by manufacturers and used according to the manufacturers' instructions. Packaging systems for heat sterilization should permit heat penetration and direct contact with item(s) surfaces. Prior to use in sterilizing items, packaging materials should be maintained according to the manufacturer’s recommended storage conditions.

Method of Wrapping/Protecting the Item
The appropriate size wrapping material should be selected to achieve adequate coverage of the item being packaged. The item should be wrapped securely to prevent gapping, billowing, and air pockets from forming, conditions which may adversely impact the sterilization process. Sequential wrapping using two barrier-type wrappers is a common technique employed to provide an additional barrier to microbial contamination and to facilitate introduction of the item into aseptic processes. Peel packages or pouches are commercially available pre-sterilization wraps, and should be used according to the manufacturer’s instructions. For items that cannot be enclosed in a package, all openings or areas requiring barriers must be completely and securely covered. Wrapping methods should be standardized as detailed instructions in Standard Operating Procedures (SOPs).
(Continued from page 14)

Transport/storage Conditions
Terminal steps for some sterilization processes require consideration of holding conditions. For example, cool-down of items subjected to steam sterilization should be conducted in stringently classified areas (e.g., Grade C or better). The environment of areas through which the sterilized materials are transported may require consideration. A contiguously wrapped item is far less likely to be compromised than an item with openings that have been wrapped or covered. Enclosed, classified (HEPA-filtered) transport chambers are commonly employed as a protective measure. Storage areas should be of cleanroom classification appropriate to the items’ intended uses. Shelving and storage space should be of a design that facilitates “first in-first out” usage and minimizes excessive movement of items by personnel. All transport and storage practices should be standardized as detailed instructions in Standard Operating Procedures (SOPs).

SCIENTIFIC SOLUTION

Establishment of the expiration dating for sterilized items should be accomplished via a validation process. The intent of this type of validation is to evaluate the sterile barrier after some targeted holding time (including the holding environment), and not the actual sterilization process or the sterilization equipment. Validation of both the sterilization equipment and the sterilization process of the items should be done prior to this validation process.

There are three important aspects of establishing an appropriate time limit or expiration date for sterilized items:
1. Preparation of the item for sterilization (establishment of the sterile barrier)
2. Holding environment of the item after sterilization

(Continued on page 16)
3. Holding time

**Item Preparation**

The important aspect of preparation of an item to be sterilized is the barrier that is developed between the item and the environment that it will be held in.

The type of sterile barrier (protective wrappings) along with the type of item (Equipment type) can be used to categorize the items into grouping for the validation.

Since the groups are based on similar sterile barrier types and item types, representative article(s) from each group can be selected and utilized for the execution of the validation based on the greatest risk of contamination with respect to that particular configuration of sterile barrier and equipment type. The representative article can be used to assess and validate a hold time for entire groups that have the same type of wrapping and are held in the same type of environment. Each representative article should be tested in triplicate.

**Holding Environment**

Sterilized items may be held for the duration of validation period in either similar environmental conditions to what the equipment would routinely be held under, or exposed to a worst-case condition. Transfer of items from their sterilization processing area to holding areas and to final usage areas should be simulated.

**Holding Time**

Targeted Holding Times should be developed for the validation. The times selected need to be at a minimum, a representation of the typical time that the routine process will require. It would be advantageous to add a safety factor to that time to ensure the maximum amount of time potentially required by the process. Another option would be to evaluate the maximum time possible. This could be accomplished during the validation by preparing, sterilizing, and holding enough items in order to perform the testing at selected intervals until failures are obtained, then focusing additional validation on that failure point.

**Test Method**

The test method must be able to evaluate the step mentioned above.

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**Errata**

In last month’s listing of interesting and useful articles for the QC Microbiologist we omitted:

Cundell, AM et al. 2010. Equivalence of Quality Control Strains of Microorganisms Used in the Compendial Microbiological Tests: Are National Culture Collection Strains Identical?

*PDA J Pharm Sci Tech* 64(2):137-155
rility of the items after being held in the holding environment for the targeted holding time.

Two types of testing that could be utilized to evaluate the expiration dating of sterilized items are Aseptic Processing Simulations or Direct Testing.

1. Aseptic Processing Simulations (APS):
   In this option, the sterilized items would be held to their targeted expiration date and then used in an Aseptic Processing Simulation (Media Fill). This, however, has inherent complications, in that, if you end up with a contaminated unit, tracing that contamination back to a specific item is extremely difficult.

2. Direct Testing:
   The other option is to directly test the item for sterility after it has reached its targeted expiration date. This can be accomplished by exposing the item directly to media, then assessing the media for sterility per current USP <71>. Since this option would give a more direct assessment of the sterility of the item, it is the recommended option. There are two methods to accomplish direct testing of the items, based on the type and configuration of each item: Direct Transfer Method and Fluid Path Method.

Direct Transfer Method is performed by immersing the test articles directly into a liquid nutrient medium (e.g., Soybean-Casein Digest Broth). This method is used for small articles that can be immersed in a liquid medium. These types of items are typically enclosed in a bag or pan. This method can also be used for types of wrappings where the test article, in a completely enclosed wrapping, is too large to be immersed into media. Since this method tests the type of wrapping and not the actual item inside the wrapping, the actual item that is too large for immersion can be replaced with items that can be immersed into a liquid medium. For example, if the wrapping is a bag that is sealed with autoclave tape, then the sterile barrier created by the bag and the taping is being tested. So, stoppers, coupons, vials, or other similar items that can be easily manipulated and immersed into media may be substituted into the bag in place of the actual item.

After being sterilized and held in the holding environment for the specified holding time, the test article(s) (either actual item(s) or representative item(s)) are aseptically transferred into a sterile container containing the incubation medium. The item(s) should be completely immersed. The sterile container containing the media and the test article(s) is then incubated and tested for sterility.

Fluid Path Method is performed by wetting the interior surface of an article with a liquid nutrient medium, then testing the medium for sterility. This method can be used for articles that have a usual “path” for fluid. Examples of these types of items are tanks, bottles, and transfer tubing.

The volume of the test article should be taken into consideration. In some cases it may be possible to collect and incubate a test sample that is equal to or greater than the volume of the item or of the fluid path. If that volume is too large, a representative volume could be established for the validation. The following is an example of volumes that could be evaluated:

- Minimum of 10% of interior volume for articles with volumes greater than 10L.
- Minimum of 50% of interior volume for articles with volumes less than or equal to 10L.
All medium used in this testing should be sterilized using a validated sterilization process. The medium must be tested for suitability (growth promotion and sterility of medium). The qualification of the sterility test method should be performed, as described in current USP <71>.

Samples collected for sterility testing are incubated for a minimum of 7 days at 20-25°C and then for a minimum of 7 days at 30-35°C (per current USP <71>).

CONCLUSION

Current industry and regulatory guidance for validation of sterile barrier configurations and hold times, within the context of the sterilization method, is currently minimal, due largely to the various types and configurations of components and equipment. However, a firm should approach this validation with the goal of standardizing packaging methods and validating hold times, and reflect the outcome in detailed SOPs. Additional research is necessary to develop data in support of common industry practices in these areas, which can in turn be extrapolated to firm-specific variations. Research specific to closure materials (ex. cloth or paper wrappings) and methods (ex. sealing), and their ability to establish a sterile barrier needs to be formally recognized and experimentally determined though industry wide focus groups utilizing organizations such as ISPE, PDA, etc. to develop common best practices. These studies should consider and normalize event, time, and location factors for ensuring the sterility of containers and closures.

REFERENCES

About the Authors:

Robert Westney is currently President and Director of Quality and Operations for Cryologics, Inc. He is also Principal Consultant for Westney & Associates Consulting, LLC. He has nearly 25 years of experience in the GMP industry, including Quality Control Microbiology, Quality Assurance and Regulatory Affairs. He holds a Master of Science degree from Temple University in Quality Assurance/Regulatory Affairs. He is Regulatory Affairs Certified (RAC), and is a Certified Manager of Quality/Organizational Excellence (CMQ/OE).

Eric Staib is currently Director of IT Quality, Global Computer Systems Validation, at Covance Inc. He has 13 years of pharmaceutical industry experience in various GXP areas including direct experience in quality systems management, microbiology, quality engineering, information technology, and laboratory operations. He holds a Bachelor of Science degree from James Madison University in Biology, a Master of Science degree from Temple University in Quality Assurance/Regulatory Affairs, a graduate certificate from Lehigh University in Project Management, and is currently finishing a MBA from Drexel University in Pharmaceutical Management.

Mark Fosbenner is currently Senior Manager of Regulatory Affairs, CMC Facilities, at Novartis Vaccines and Diagnostics. He has 18 years of experience in the GMP industry, including experience in Water Systems Engineering, Consulting, Computer System / Equipment / Facility Validation, Sterility Assurance, and Regulatory Affairs. He holds a Bachelor of Science degree from Albright College in Theoretical Physics, and a Master of Science degree from Temple University in Quality Assurance/Regulatory Affairs.

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Any questions concerning USP documents should be sent to Radhakrishna (Radha) Tirumalai, Ph.D. You can reach Dr. Tirumalai at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at RST@USP.org. You can write representing your company, or as an individual scientist.

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