Another Year Starts

2009 starts off with an aggressive schedule for the PMF. In addition to more conferences than we have ever scheduled before, we have also branched off into benchmarking activities. These activities have been a popular activity of both the PMFList and the PSDGList and in 2009 we will be moving to perform systematic surveys to benchmark different aspects of the pharmaceutical industry for our membership. Please let us know if you would like to participate.

In the first article of this issue we have a review of bioburden as a product concern by Jobair Hossain from Bangladesh. This review stresses the role microbiology plays in product quality and provides an excellent overview of testing methods commonly used in the QC laboratory.

Russ Nyberg contributes a second article on 3rd Party verification of biological indicators (the first appeared in the May, 2006 issue of PMF Newsletter). The practice of confirming D-values is not required for the end-user and is not recommended except under specific conditions (see USP chapter <1035>). Russ Nyberg discusses aspects of this practice, and provides valuable insight into the associated pitfalls.

Finally, the interested reader can find brief descriptions of the upcoming PMF Puerto Rico Sessions at the end of the newsletter.

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Importance of the Bioburden Test in Pharmaceutical Quality Control

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Introduction
Bioburden is defined (for the purposes of this discussion) as the presence of microbial contaminants in pharmaceutical raw material and finished product of Pharmaceuticals Industries. This microbial contamination may be bacteria, fungus and/or viruses depending upon the types of formulations.

Bioburden testing is used to estimate the number of microorganisms/contaminants in or on product prior to sterilization; solid components and process aids; liquid components and process aids; chemical raw materials and gaseous components.

Methods and Principles
There are some basic considerations important to adherence to Good Laboratory Practice (or cGMP, depending on the region) and general laboratory principles for bioburden testing:

1. It should be assured that the method of sample preparation does not result in transfer of antimicrobial substances. This can be demonstrated by seeding relevant media or rinse fluids with known numbers of standard fertility test microorganisms (ATCC or other national stock culture strains are recommended for control strains) known to be sensitive to substances likely to be present. After appropriate contact time the numbers of survivors can be determined.
2. If antimicrobial substances are present, they should neutralize or removed by suitable method (e.g., filtration, dilution, neutralizing agent). It is necessary to perform a suitability test for the method to be used to demonstrate this neutralization. This is performed by seeding the material with known levels of indicator microorganisms and determining the number recovered by the chosen recovery methods and shown to be equivalent to the number of organisms inoculated into the material.
3. Techniques of recovery should also facilitate both the required nutritional and physical requirements of the microorganisms to be isolated. Methods should be always validated against controlled organisms.

Sampling
Sampling is an important and vital point in bioburden testing. It should always be kept in mind that a faulty sampling may produce a biological false positive result. That’s why before sampling equipment and apparatus should be properly sterilized and labeled. When sampling containers are prepared with neutralizing agents (e.g., sodium thiosulphate and others) this procedure should be properly validated and controlled. Raw and packaging material should be always collected in an aseptic manner (e.g., HEPA filtered laminar air flow cabinets may be useful).

(Continued on page 3)
Utilities are another source of bioburden. Water samples should be collected after allowing free flow of water from the outlet for at least 2 minutes. Steam samples should be collected aseptically, allowed to condense and also tested as water. If the interval between sampling and test is greater than 4 hours, the samples should be kept in controlled temperature (2°C to 8°C). These samples should not be stored longer than 24 hours before testing. The quantity and the amount of samples should be collected as per as compendia or regulatory guideline.

Finally, bioburden testing can and should be used as an in-process monitoring method. In the case of terminally sterilized product, pre-sterilized samples should be collected from the very first and last filled as a minimal sampling plan.

**Different Aspects of Bioburden Test**

Contamination possibilities of pharmaceuticals – Potential microbial contaminants in finished product can result in a product recalls. For example:

- Sedimentation of syrup – Microbial contaminants can result in sedimentation of the syrup in some cases, even though products might contain preservatives. This is a particular issue when the microbial contaminants are elevated.
- Physical changes in tablets & capsules – Physical changes may result from the presence of microbial contaminations. Higher microbial counts can result in the degradation of the coating material and even the liquefaction of the capsule shell in certain cases.
- Turbidity of sterile solution – Even the presence of a single viable microbial cell in sterile solution might result in the contamination and turbidity of the product (depending on the formulation).
- Shock syndrome due to endotoxin – Presence of gram negative bacterial endotoxin in the products may be at levels high enough for patient toxicity (in some cases 10^4 cfu/ml of gram negative bacteria is pyrogenic). This does not require viable cells to be present.

**Sources of Microbial Contamination in Pharmaceutical Products**

Bacterial contamination can come from a variety of sources:

External sources of contamination are plentiful. Examples can include excipients, containers, packag-
schedule and ensure that it meets minimum requirements for the quality standards established.

Air circulation system (HVAC) - Air born microorganisms also play an important role in the increase of bioburden in pharmaceutical preparations. It is necessary to maintain and monitor a proper air circulation system in the manufacturing area and to maintain control of the environment through HEPA-filtered source air and air pressure balancing to ensure outward movement of clean air from the fill area outwards.

Personnel - Operators are the single largest potential source in increasing the bioburden load in pharmaceutical preparation. Ill working personnel may shed pathogenic microorganisms in the product during working with it, and even healthy individuals are enormous sources of indigenous bacteria. It is essential to monitor the personnel hygiene of the working personnel’s and train them in the proper use of personal protective gear (PPE) and procedures to minimize product contamination.

Maintenance and cleaning etc. - Lack of proper maintenance a cleaning may increase bioburden load in the pharmaceutical preparation. All cleaning procedures should be validated to be efficacious against bacterial, mold and spores present in the facility.

Factors Affecting the Survival and Growth of Microorganisms in Products -
Nutritional contents of the products - Product formulations high in proteins and minerals have the potential to support the growth of microorganism. Even products with minimal nutrients can support microbial growth if not protected with an effective preservative system.

Storage condition - Storage temperature, available water, light, etc can have an important role in the potential for microbial growth.

Chemical properties - osmolality, pH, available water etc can have a great effect on the growth and survivals of the contaminants.

Spoilage of products/Impact on Stability
Microbial breakdown of the products will result in their spoilage. This can have cosmetic as well as quality effects on the stability of the product.

Infection Hazard from Contaminated Products -
The major concern from contaminated products is, of course, pathogenic microorganism contamination leading to patient illness. This can occur via:
- Gastrointestinal Infection Through contaminated oral Products.
- Eye infection from contaminated eye drops.
- Skin infection from contaminated cream & ointment.
- Septicemia from contaminated IV fluids & injections.
- Toxic shock from contaminated IV fluids & injections.

Note that the compendial requirements for the Microbial Limits Tests does not demonstrate the absence of pathogenic organisms, only those specific ones of the test. Further testing is needed to demonstrate absence of objectionable organisms.

Sartorius is an internationally leading laboratory and process technology supplier covering the segments of biotechnology and mechatronics. Sartorius has over 75 years experience in the manufacturing of cellulose nitrate membranes which are routinely used today for microbiological analysis.

The detection of microbial contamination in sample liquids such as final product, incoming inspection or during in-process testing plays a significant role in the quality assurance process. The requirements for a practical microbiological test method are that it permits quantitative and reproducible detection of trace contamination and that it can be performed efficiently and economically under routine conditions. These requirements are fulfilled optimally by the membrane filtration method. The membrane filter method is worldwide accepted and the preferred method for analyzing aqueous solutions for microbial contamination.

Sartorius offers an extensive line of high quality and reliable membrane-based solutions for all your microbial analysis needs, specifically for microbial enumeration, sterility testing and air monitoring. To learn more about Sartorius Microbiology Products, please visit us at: www.Sartorius.com/microbio.

The PMF Newsletter thanks Sartorius for its sponsorship.
Current Regulatory Requirements & Specifications -
Although the compendia are due to be harmonized in a short time, there are some interesting differences in the current bioburden expectations.

**BP**—Microbiological Quality of Pharmaceutical Preparations -In the manufacture, packaging, storage and distribution of pharmaceutical preparations, suitable means must be taken to ensure their microbiological quality. The pharmaceutical preparations should comply with the criteria given below (see table)

**USP**- United States Pharmacopoeia does not categorize the products like BP. It specifically define the limits of total viable bacterial and fungal count for each product separately for per g or per ml.

**EP**- Regulatory requirement of EP is quite most similar to that of BP.

At present the US Pharmacopoeia (USP), the Japanese Pharmacopeia and the European Pharmacopoeia are in the final stages of harmonization. The finalized versions of these tests will be in effect this year, and have been discussed previously in the newsletter (see http://www.microbiologyforum.org/news.htm).

<table>
<thead>
<tr>
<th>Category</th>
<th>Types of Preparation</th>
<th>Total Viable Aerobic Count</th>
<th>Absence of Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1</td>
<td>Sterile products</td>
<td>Sterile</td>
<td>Sterile</td>
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</table>
| Category 2 | i)Preparation for topical use and for use in the respiratory tract.  
ii)Transdermal Patches | i) 10^2 micro-organisms (Aerobic bacteria and fungi) per g, per ml, per patch. | 1) Absence of Pseudomonas aeruginosa on 1 g or 1 patch. 
ii) NMT 10^1 enterobacteria and certain gram-negative bacteria per g or ml.  
iii) Staphylococcus aureus on 1 g or 1ml |
| Category 3 | A. Preparations for oral and rectal administration.  
B) Preparations for oral administration containing raw materials of natural (animal, vegetable or mineral) origin for which antimicrobial pretreatment is not feasible. | A. NMT 10^3 bacteria and 10^2 fungi per g or ml.  
B. NMT10^4 bacteria and 10^2 fungi per g or per ml | A. Absence of Escherichia coli on 1g or 1ml.  
B. NMT 10^2 enterobacteria and certain other gram negative bacteria per g or per ml  
-Absence of Salmonella In 10g or 10ml.  
-Absence of of -Escherichia coli on 1g or 1ml.  
-Staphylococcus aureus on 1 g or 1ml. |
| Category 4 | Herbal medicinal products consisting solely of one or more herbal drugs ( Whole, reduced or powered )  
A. Herbal medicinal products to which boiling water is added before use.  
B. Herbal medicinal products to which boiling water is not added before use. | A. -NMT NMT10^7 bacteria and 10^5 fungi per g or ml.  
B. NMT NMT10^5 bacteria and 10^4 fungi per g or ml. | A. - NMT10^6 of Escherichia coli on 1g or 1ml using suitable dilutions.  
B. -NMT 10^23 entero-bacteria and certain other gram negative bacteria per g or per ml.  
-Absence of of -Escherichia coli on 1g or 1ml.  
-Absence of Salmonella In 10g or 10ml. |
Methods of Microbial Contamination Detection -

Sterility Test

a) Direct Inoculation of the culture medium - Transfer the quantity of the preparation to be examined according to the compendia specifications directly into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed.

- If the product to be examined has antimicrobial activity, the test is carrying out after neutralizing with suitable neutralizing substances or by dilution in a sufficient quantity of culture medium.
- When it is necessary to use a large volume of the product it may be preferable to use a concentrated culture medium prepared in such a way that it takes account of the subsequent dilution.
- Where appropriate, the concentrated medium may be added directly to the product in its container.
- Diluents commonly used in membrane filtration are 1 g/L neutral solution of meat or casein peptone, sometimes containing a suitable emulsifying agent such as Polysorbate 80 at a concentration of 10 g/L.
- Incubate the inoculated media for not less than 14 days.

Catgut and other surgical sutures for veterinary use - For each medium use not less than the quantities of the product according to regulatory or compendia requirement. Use sufficient medium to cover adequately the material to be tested (20ml-150 ml).

Test for Microbial Contamination/Microbial Limit Test

Total viable aerobic count

- These tests will allow quantitative analysis of mesophilic bacteria and fungi, which may

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grow under aerobic conditions.

- The tests are designed primarily to determine whether or not a substance that is subject of a monograph in the Pharmacopoeia complies with the microbiological requirements specified in the monograph.
- Carry out the determination under conditions designed to avoid accidental contamination of the product to be examined. The preparations taken to avoid contamination must be such that they do not affect any micro-organisms which are revealed in the test.
- If the product to be examined has antimicrobial activity this must be adequately neutralized.
- The choice of a method may be based on factor such as the nature of the product and the expected number of microorganisms. Any method, which is chosen, must be properly validated.

**Determine the total viable aerobic count by:**

**Membrane Filtration:**

Use membrane filters having a normal pore size not greater then 0.45µm and 50 mm in diameter-whose effectiveness to retain micro-organisms has been established.

- Cellulose nitrate filters are used for aqueous, oily and weekly alcoholic solutions and cellulose acetate filters for strongly alco-holic solutions. The filtration apparatus is designed to allow the transfer of the filter to the culture medium.
- Transfer suitable amount of the sample prepared as requirements of sample preparation to two each of two membrane filters and filter immediately.
- Wash each filter with not more than 300 ml of buffered sodium chloride–peptone solution pH 7.0 which may contain surface–active agents such as polysorbate 80 or inactivators of antimicrobial agents.
- Transfer one of the membrane filters, intended primarily for the enumeration of bacteria, to the surface of a suitable agar medium such Tryptic soya agar and other intended primarily for the enumeration of fungi to the surface of the Sabouraud Dextrose agar. Incubate the plate for tryptic soya agar at30°C-35°C, and Sabouraud Dextrose agar at 20°C-25°C for 5 days.
- When examining transdermal patches, filter 50 ml solution.
- Select plates with the highest number less than 300 colonies (100 colonies) and calculate the number of colony forming units per gram or milliliter of product.

**Plate Count Methods**

Pour-plate Method: - Using Petri dishes 9cm in diameter, add to each dish 1 ml of the sample prepared as regulation of sample preparation and 15-20 ml of liquefied agar medium of Tryptic soya agar for the cultivation of bacteria and Sabouraud Dextrose agar for the cultivation of

(Continued on page 8)
fungi at not more than 45°C. Prepare for each medium at least two Petri dishes for each level of dilution.

- Incubate the plate for Tryptic soya agar at 30°C-35°C for bacteria, and Sabouraud Dextrose agar at 20°C-25°C for fungi for 5 days.
- Select plates with the highest number of colonies less than 300 colonies (100 colonies for fungi) and calculate the number of colony forming units per gram or milliliter of product.
- Take the arithmetic average of the counts and calculate the number of colony forming units per gram or milliliter.

Surface or Spread-plate Method: - Using Petri dishes 9cm in diameter add to each dish 15-20 ml of liquefied agar medium of tryptic soya agar for the cultivation of bacteria and Sabourauds Dextrose agar for the cultivation of fungi at not more than 45°C to each Petri dish and allow it to solidify. Dry the plates in LAF or in an incubator. Spread 0.1 ml of the sample prepared as per as regulatory requirements of the sample over the surface of the medium. Use at least two Petri dishes for each medium and each level of dilution.
- Incubate the plate for tryptic soya agar at 30°C-35°C for bacteria, and Sabourauds Dextrose agar at 20°C-25°C for fungi for 5 days.
- Select plates with the highest number of colonies less than 300 colonies (100 colonies for fungi) and calculate the number of colony forming units per gram or milliliter of product.
- Take the arithmetic average of the counts and calculate the number of colony forming units per gram or milliliter.

**Most Probable Number Method (MPN)**

This method is reserved for bacterial counts when no other method is available.

- Prepare the series of at least three subsequent tenfold dilutions (1g, 0.01g, 0.001g) of the product as per as compendia requirements for the sample preparation. From each level of dilution three aliquots of 1g or 1 ml are used to inoculate three tubes with 9 ml to 10 ml of tryptic soya broth. If necessary surface-active agents such as polysorbate 80 or an inactivator of antimicrobial agents may be used. Thus if three levels of dilution are prepared nine tubes are inoculated.
- Incubate all tubes for five days at 30°C-35°C
- Record for each level of dilution number of tubes showing microbial growth.
- Determine the most probable number of bacteria per gram or milliliter of the product to be examined using MPN table.

2. Test for specified microorganisms:
- In general, these tests are looking for specified organisms, considered to be indicative of problems in cleanliness or manufacturing.
- The first step in all is an enrichment step involving incubation in rich media, to generate large numbers of organisms and resuscitate injured organisms. If the product to be examined has antimicrobial activity this must be neutralized to allow growth.
- Secondly, certain selective media is used. Select-
Differential media may also be used—these allow identification of colonies of interest based on morphological characteristics (colony size, shape, color, effect on media, etc.)

Many of the tests also require a confirmatory test using an established microbial identification method to minimize false positives.

Method Validation

Grow the Bacterial test strains separately in containers containing casein Soya bean digest broth at 35-37°C for 18 hrs. Mold strains should be grown separately on Sabouraud dextrose medium at 20-25°C for 48 hrs, and the yeast *Candida albicans* should be incubated at 20-25°C for 7 days.

Use buffered sodium chloride–peptone solution pH 7.0 to make reference suspensions containing about 100 cfu per ml.

Use the suspension of each of the microorganisms separately as a control of the counting methods, in the presence and absence of the product to be examined.

When testing the membrane filtration method or the plate count method, a count of any of the test organisms differing by not more than a factor of five from the calculated value from the inoculums is to be obtained.

When testing the Most Probable Number (MPN) method the calculated value from the inoculums is to be within 95% confidence limits of the results obtained.

To test the sterility of the medium and the aseptic performance of the test carry out the method using sterile sodium chloride–peptone solution as the test suspension. There must be no growth of microorganisms.

Validation of the filtration method

- Accurately weight 10 g or 10 ml of the product to be tested into 100 ml of buffered sodium chloride–peptone solution homogenize.
- Transfer the contents of the container to each of two membrane filter containing an inoculum’s of a small number of viable microorganisms (not more than 100 CFU) and filter immediately.
- Wash the filter with washing solution which may contain surface-active agents such as polysorbate 80 or inactivators of antimicrobial agents.
- Transfer one of the membrane filter into Casein Soya bean digest agar intended for bacteria and Sabourauds dextrose agar for fungi. Incubate the plates for bacteria at 30°C-35°C and for fungi incubate at 20°C-25°C for 5 days.
- If clearly visible growth is observed count the number of colony formed.
- The test can be carried out further modification.

Microbial Identification

Identification can be done on the basis of phenotypic and/or genotypic characteristics of microorganisms. Phenotypic methods use physical characteristics of the bacteria for identification, genotypic use genetic characteristics.

(Continued on page 10)
Automated Identification Systems

The most widely used automated phenotypic enumeration systems are BioMerieux, Vitek and Biolog System.

Vitek

The Vitek System is widely used across the pharmaceutical industry as it provides rapid, straightforward and objective identification has a large sample handling capacity, and can offer considerable labour savings in sample preparation data analysis and reporting. Viteck can provide results in the majority of cases between 8 to 15 h.

It is comprised of 30-well identification cards; a filter/sealer unit; a reader/incubator unit; a data terminal and printer. Sample preparation is simple, and involves sub-culturing isolates to obtain pure culture followed by Gram staining, oxidase, catalase or coagulase testing and suspension in saline. The well of the appropriate identification card are then vacuum-filed with saline suspension, and the filled cards are inserted into the reader/incubator.

Up to 480 cards can be analyzed, the cards being read at hourly intervals by sensors looking for changes in turbidity or colour; the reaction pattern is analyzed by computer.

Information is compared with a principal database called the Primary React File (PRF), and identification is made on the basis of this comparison.

If a result is not possible using PRF, a second database-The Secondary React File (SRF) is used and SRF is particularly useful for the identification of industrial environmental isolates.

Biolog

In the Biolog, carbon utilization produces colour changes describing a metabolic fingerprint, the identification being made by compression with an extensive database. It is of two types - either manual or automated.

The operation is relatively simple. Microbial saline suspension is prepared and inoculated into individual well of microplate containing a verity of carbon substrates. The plate is then incubated at 30-35°C, with readings being taken after a minimum of 4 h, either manually or by using the microplate reader. Bacterial identification is performed by carbon-source oxidation which is detected when increased cell suspension leads to irreversible re-

duction of a tetrazolium dye, resulting in a purple color change. The yeast test plate contains both assimilation and oxidation test. The resulting colour patterns must be analyzed by software, and identification determined after comparison with the database which encompasses most known human pathogens and major industrial environmental isolates. The results usually obtained between 4 and 18 h.

Advantages of Biolog include easy sample preparation and handling, objective identification and manual or automated sample reading facilities; most importantly, the database includes a wide range of clinical and environmental isolates.

Gas Chromatography

Gas chromatography (GC) offers an alternative to sub-
strate-utilizing identification system. Identification follows the extraction of cellular fatty acids which are analyzed by GC and compared with a central database. Fatty acid composition is a useful tool for identification due to its highly conserved nature within a taxonomic group.

The MIDI Microbial Identification System (MIS) is automated GC-based identification systems which can analyse more than 300 fatty acid methyl esters ranging in length from nine to 20 carbons.

GC analysis requires trained operators and involves the use of complex sensitive equipment. Moreover culture conditions need to be carefully controlled in order to provide reproducible fatty acid profile. Solvent extraction of cellular fatty acids is also a lengthy and multi-stage procedure. It is, however very dependable. The software system allows the user to generate database of frequently encountered isolates, and this is particularly useful when isolates are difficult to identify.

Cluster analysis of data is also possible which generates denograms (nearest neighbor plots) or two-dimensional plots for comparative analysis of isolates—a very useful method for tracking the source of contamination during troubleshooting investigation.

Genotypic Methods
Genotypic microbial identification methods based on nucleic acid analysis may be less subjective, less dependent on the culture method, and theoretically more reliable because nucleic acid sequences are highly conserved by microbial species. These methods would include DNA-DNA hybridization, PCR, 16S and 23S rRNA gene sequencing, and analytical ribotyping.

These methods may be more technically challenging for the pharmaceutical microbiology personnel and are more expensive in terms of both equipment and current testing cost and they often rely on a technology marketed by a single company. Therefore, their current use may be better suited to critical microbiological investigations associated with direct product failure or with the identifications conducted in a specialized research oriented laboratory within a pharmaceutical company or sent to a contract testing laboratory.

Conclusion
The bioburden test is very important to enumerate and identify objectionable microorganisms in Pharmaceutical products. To ensure safety and to assure quality there is no alternative to this test.

REFERENCES
Biological Indicators, D-Value and Population Verification/Assessment

Russ Nyberg  
Director of Technical Support,  
Biological Indicator Manufacturing Facility  
Raven Labs, Omaha, Nebraska  
(a division of Mesa Laboratories, Inc.)

A large number of end-users of Biological Indicators (BIs) are sending the BIs out to 3rd party laboratories for verification of the D-Value and/or Population of the BI prior to acceptance for use. Having worked within the biological indicator manufacturing industry for nearly 16 years and through hundreds of personal conversations with end users of BIs in regards to BI verification/assessments, it becomes increasingly clearer to me that some very real confusion exists in relation to 3rd party testing and assessment/verification testing results and what can and can not be done with this data.

USP 31, ‘User’s Responsibility’ states, “The user should establish in-house acceptance standards for biological indicator lots and consider rejection in the event the biological indicator lot does not meet the established in-house performance standards. A Certificate of Performance should be obtained for each lot of indicators, and the user should routinely perform audits of the manufacturer’s facilities and procedures.”……… “Upon initial receipt of the biological indicator from a commercial supplier, the user should verify the purity and morphology of the purchased biological indicator organisms. Verification of at least the proper genus is desirable. Also, a microbial count to determine the mean count per biological indicator unit should be conducted. The manufacturer’s comments relative to D-value range, storage conditions, expiration dating, and stability of the biological indicator should be observed and noted. The user may consider conducting a D-value assessment before acceptance of the lot.” (1)

In review of the above USP excerpt, many facilities have instituted in-house acceptance criteria for in-coming BIs prior to allowing them to be used. “….. a microbial count…should be done.” A large number of end users are doing this. They are performing the population assay’s themselves or are sending them out for 3rd party population verification. When D-Value is concerned, most facilities do not have the proper equipment to do a D-Value assessment so they are sending these BIs off to a 3rd party for testing. USP states that… “The user may consider conducting a D-value assessment before acceptance of the lot.” One should note that ‘may consider’ is not a must or should. The statement is fairly clear, may consider. If one chooses to consider a D-Value assessment or the facility protocol requires a D-Value assessment prior to use, then a 3rd party testing lab is usually needed. When the test BIs are sent off to the 3rd party testing lab, the end-user should be requesting a D-Value assessment using the same D-Value testing methods used initially by the BI manufacturer to determine and certify the D-Value for that particular Lot of BIs. The methods used by the BI manufacturer should be stated on the certificate of analysis (C of A) provided with the BI Lot. It is important to remember that in a D-Value assessment test, you are getting from the 3rd party assessment lab just that, an assessment. An assessment is not the same as a determination.

If the assessment is within the allowable + or – 20% of the certified D-Value stated on the manufacturers certificate of analysis, the assessment passes the test and the D-Value ‘as certified’ on the C of A can be accepted and is to be used whenever that Lot of BIs is used. The certified D-Value has been assessed, substantiated, supported or confirmed. Regardless of the terminology used, it is still an assessment. However, it is not to replace the manufacturers certified D-Value. Unfortunately in far too many cases this is what is happening. The end user now uses the 3rd party testing labs result for D-Value (or population) as the ‘new established D-Value’. This just can not be done. (Continued on page 13)
The D-Value testing assessment cannot replace or be used to re-label the certified D-Value determined by the manufacturer. To initially determine and certify the manufacturer's stated D-Value, methods allowed by USP (2) and ISO (3) were used. As in ISO 11138 series, to obtain a label claim or certify a determined D-Value, one must use two of the three methods outlined in ISO and USP: Most Probable Number method by direct enumeration, a Fraction Negative Method (such as Spearman/Karber) or Survive/Kill. Whichever two methods are chosen, two of the three must be used for D-Value determination. A 3rd party assessment is not a determination and may not in any way be used to re-label a BIs resistance characteristic certified by the manufacturer.

A similar situation exists with 3rd party population verification. For the BI to pass the population verification, the population result needs to be within +300% and -50% of the labeled population. If the population being verified falls within this range, the requirements of the test are met and the population has been verified. However, the verified population is not the new certified population to be used in further studies or validations done with this Lot of BIs. In most situations, BI manufacturers are much more familiar with, have validated and have extended experience in performing population assays on their particular BIs. Having a 3rd party labs assay result replace a manufacturers certified population, even if only slightly different than the C of A listed population makes little sense and is simply not to be done. The bottom line is that 3rd party verifications are only that…verifications and are not in any way intended to replace a manufacturer’s certified BI characteristics such as D-Value or population. Some testing labs are actually performing re-certifications and some end-users are using the 3rd party verification to replace the C of A labeled values. If verification criteria are met, the certified characteristics on the manufacturers C of A are to be used. I certainly would not want to be the one who re-labeled a medical device for use in my facility if I were the end-user of a purchased BI. The FDA may even consider this adulteration of a medical device.

References
1. USP. 2008 <1035> Biological Indicators for Sterilization USP 31, General Information, pg. 401
2. USP, 2008 <55>, Biological Indicators-Resistance Performance Tests.
3. ISO 11138-1 Sterilization of Health Care Products, Biological Indicators Part 1.
## Upcoming Events

**March 30-31** PMF 2009 Conference on Environmental Monitoring in Philadelphia, PA  

**April 20-22, 2009** PMF Puerto Rico Microbiology Sessions in San Juan, Puerto Rico  
- GMP in Microbiology **April 20**  
- Validation of Microbiological Tests **April 21**  
- MDD and Investigations **April 22**  

**May 18-19, 2009** PMF Conference on Microbial Investigations in Philadelphia, PA

**June 1-4, 2009** CLAMME Conference on Pharmaceutical and Cosmetic Microbiology (Buenos Aires, Argentina)  

**June 8-9, 2009** PMF Conference on Management of the Microbiology Function in a Regulated Industry in Philadelphia, PA

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### Discussion List Update

**PMFList:**  
Number of Subscribers: 3347  
Number of Countries: 88  
Number of Messages Last Month: 165

**PSDGList** (Pharma Stability Discussion Group):  
Number of Subscribers: 1098  
Number of Countries: 34

Membership is FREE. To **join the PMFList**, visit [http://microbiol.org/pmflist.htm](http://microbiol.org/pmflist.htm) and register.

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### USP Corner

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.