



MICRO NEWS

VOLUME 2/2004

Occasionally a radiation validation study will give undesirable results. These results are often in the form of additional positives in the sterility test, hidden bioburden which is not extracted using standard bioburden methods, or unique products which do not fit in the typical methodologies. In these cases we have experts who can help.

Recently, we have made significant additions to our Sterilization website section by adding to our Radiation Sterilization web pages. Under the new pages you will find excellent resources, including:

- Which radiation sterilization method do I use?
- Failures: My sterilization validation or dose audit failed, now what?
- Glossary of terms
- Several study outlines including AAMI Methods 1 and 2, and VDmax TIR 27
- A complete test list for radiation validations

See more at
<http://www.nelsonlabs.com/irradiate.htm>

We hope you continually find the information, study outlines and other resources on the Nelson Labs website (www.nelsonlabs.com) useful and educational. Should you have questions about your radiation sterilization validation, dose audit, failure investigations, bioburden determinations, or specific AAMI requirements, please contact me.

Martell Winters, B.S. SM(NRM)
Technical Sales (☞ Radiation Sterilization Guru)
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WEBSITE UPDATES

RADIATION STERILIZATION

For general comments about our newsletter or website, please contact John Bolinder at jbolinder@nelsonlabs.com or Mike Pizzi at mpizzi@nelsonlabs.com

The Radiation Team at Nelson Laboratories specializes in determining the doses required for the sterilization of product, as well as the testing of irradiated product. Nelson Labs scientists are actively involved on the AAMI Microbiological Methods, Sterility Assurance Level, Radiation, and Terminology working groups. We participated in the development of TIR 13409, TIR 15844, TIR 15843, TIR 27 VDmax, ST 67, TIR 17, TIR 72, TIR 29, and 11137.

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“...there was no specified evaluation for microbial contamination of product contact surfaces.”

FDA
WARNING LETTER

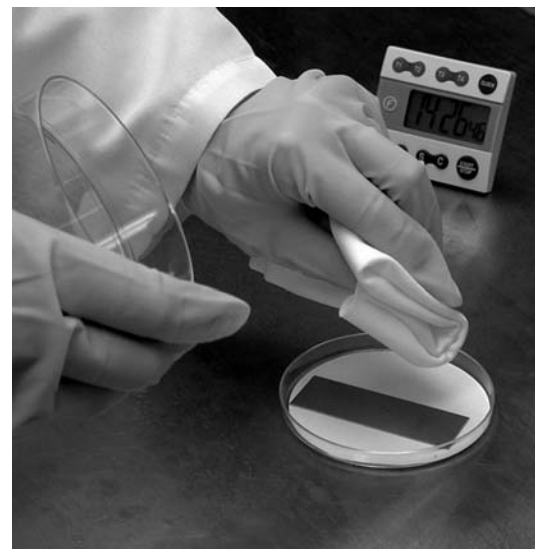
Validation of sanitizing agents for effectiveness against organisms found in cleanroom environments is an area of increasing concern to both manufacturers and regulatory agencies. Manufacturers are being held to a higher standard when it comes to product sterility. Regulatory agencies are more frequently asking for validation data to support sanitation and disinfection procedures and supplies used in cleanroom areas where organisms are routinely being found.

In addition, the FDA has issued several 483's and warning letters for items such as:

“... procedures address general cleaning but not disinfection.”

“Aseptic processing areas are deficient regarding a system for monitoring environmental conditions... there is no approved procedure for this test method.”

“... there was no specified evaluation for microbial contamination of product contact surfaces.”



SURFACE

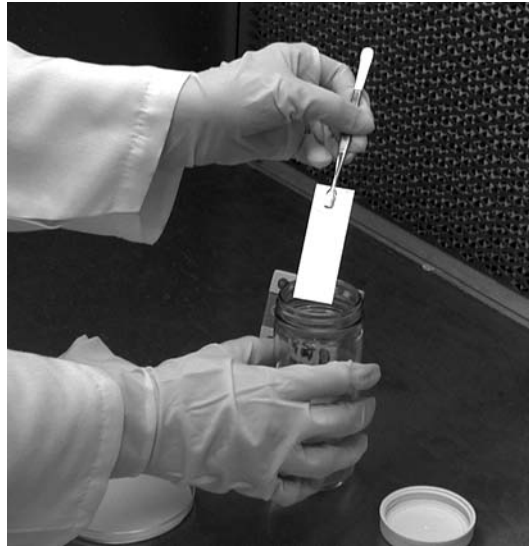
IS YOUR CLEANROOM SANITATION/
DISINFECTANT
DISINFECTION METHOD VALIDATED?
VALIDATIONS

by Bryan Wilson, B.S. RM(NRM)

Methods currently used to validate disinfectant programs include demonstration of efficacy with a kill vs. time procedure, testing after the disinfectant has been applied to a dried contaminated surface, or simulated use protocol, and shelf-life validations, which may also be verified by a kill vs. time procedure. Although, the kill time procedure is a valuable tool in determining the time necessary to achieve an acceptable log reduction of organism, it does not address the documented effects of variable

efficacy results that occur when a product is applied to different types of surfaces containing dried organism.

As no specific guidance documents are currently available for disinfectant validation testing many protocols have been based on a modified AOAC Official Method 961.02 for Germicidal Spray Products. However, a new document is now in revision under USP <1072> as a draft which not only offers guidance and background on how this test should be performed, but also gives insight as to why it should be performed.



PROCEDURE:

To summarize the <1072> method, the test coupons or surfaces are challenged with a microbial or spore suspension and then dried for 30-40 minutes at $37 \pm 2^\circ\text{C}$. Each coupon is then treated with a disinfectant product. Methods to apply the disinfectant to the coupon surface can vary greatly and are adjusted to simulate actual use conditions, such as a spray, mop, or wipe application. Some examples for application are spraying directly, wiping with a sterile towel or using a cleanroom wiper saturated with the disinfectant.

Common materials selected for testing include stainless steel (hoods, counters, etc.), polyvinyl chloride (curtain materials), and epoxy (floors). Suggestions for other surfaces are also given in the <1072> draft document.

EXPOSURE TIMES:

Another aspect to consider is the exposure time. For most general disinfectants, a minimum of 10 minutes is recommended, but not required. A 10 minute exposure time is required by the EPA for a product to receive a basic bactericidal registration; However, the amount of time required for the same disinfectant to kill a variety of organisms dried on a variety of surfaces may vary as much as 10 additional minutes, depending on the surface type and organism combination used. The exposure time should be the same time that will actually be allowed in facility procedures for items being disinfected in the cleanroom or other sterile environments.

SAMPLING METHOD:

One of the most important steps to be considered is the sampling method after the dried organism has

been challenged with a disinfectant product. According to many commonly used references, sampling methods such as swabbing, rinsing, or recovery by contact plates have been recommended in the past. Based on current literature however, recent studies have shown that these methods do not yield a high recovery of test organisms from the surface. We find that for contact plates, each plate will yield an approximate 10% recovery of the test organism dried on the surface. This being the case, we suggest these methods not be used for validation purposes, but rather for environmental monitoring once the disinfectants have been validated by a more reliable and higher yield method of recovery.

We suggest using a disinfection procedure where treated coupons are aseptically transferred to neutralizing media, which doubles as a surfactant. A total bioburden test is performed to remove any surviving microorganisms. Aliquots of the extract fluid are then plated in order to quantify the bioburden of each individual coupon. The counts are compared to untreated inoculated coupons that have been extracted in the same manner, and the reduction of the challenge organisms is measured in log and percent reductions. This method has been shown to yield a 90% recovery and up to a 99.9% recovery for some organisms. By demonstrating this level of extraction efficiency, a higher confidence and more accurate log reduction value can be obtained and reported.

ORGANISM SELECTION:

The panel of organisms selected for challenging the disinfectants may vary. Nelson Labs recommends that a spectrum of representative organisms be selected for the test including, but not limited to, Gram positive bacteria, Gram negative bacteria, fungi, mold spores, and Gram positive spore-forming

“Aseptic processing areas are deficient regarding a system for monitoring environmental conditions... there is no approved procedure for this test method.”

FDA
WARNING LETTER



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Block S.S. (ed). *Disinfection, Sterilization, and Preservation*. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2001.

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U.S. FDA Compliance Program Guidance Manual For FDA Staff: Drug Manufacturing Inspections Program 7356.002

U.S. FDA Warning letter SEA 02-18; December 13, 2001

U.S. FDA Warning letter W/L 08-03; November 19, 2002

bacteria. Additionally, environmental isolates from the facility should be tested. Typical examples of challenge microorganism isolates include *Staphylococcus aureus*, *Micrococcus luteus*, *Candida albicans*, *Aspergillus niger*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

According to the USP <1072> draft document, "Disinfectants are less effective against higher concentrations of microorganisms used in laboratory challenge tests than they are against the number found in cleanrooms." The document goes on to suggest that organisms from the log phase of growth which are used in the laboratory are more resistant than those found in the static phase or from stressed environments such as cleanrooms.

ACCEPTANCE CRITERIA:

The USP draft suggests that, "sufficient organisms need to be inoculated... to demonstrate at least a 2 (for bacterial spores) to 3 (for vegetative bacteria) log reduction during a pre-determined contact time." This type of contamination is considered worst-case as you will not typically see contamination of this magnitude in a normal cleanroom environment. There is no mention in the draft document of reductions for yeasts or molds, however, it can be deduced that a 2 to 3 log reduction would also be acceptable based on the resistance of the organisms involved.

REVALIDATION:

Some may say that once a disinfectant has been validated, a re-validation should not be necessary. However, environments are constantly changing and organisms adapting. If a new material is introduced to the environment such as new equipment, counter tops, or curtain materials the validation should be performed again using these materials. If a procedure is changed from a wipe application to a spray and wipe, or perhaps a new type of wipe is being used, the change should be validated. If higher levels of a particular organism are frequently being found during routine monitoring, it is suggested that the validation be repeated for corresponding surfaces and disinfectants with that organism.

CONCLUSION:

Now that a guidance is being considered for a surface validation procedure, the interest in sanitation and disinfectant validation data for regulatory review may increase.

From feedback received through many of our customers at Nelson Laboratories, Inc. we have also taken notice of an increased focus by regulatory agencies on data on actual organism isolates in addition to American Type Culture Collection (ATCC) strains. This trend may be associated with a desire to show efficacy on actual cleanroom organisms versus efficacy on ATCC strains that have been passed multiple times and may in some cases be weaker organisms. However, it is still recommended that some ATCC strains be used in order to establish data which can be compared to previous studies and scientific literature. The most important item to focus on is the validation procedure itself. The procedure should employ the use of all appropriate surfaces, common isolates and ATCC organisms, an appropriate recovery method (again, we recommend a total bioburden of the test coupon) and a proper neutralization verification to demonstrate the accuracy of the log reductions found. Demonstration of growth promotion with the specific lots of media and the actual test organisms should be standard practice.

Kaye Markel Retires

From delivering papers at age eight, to being the senior inspector at Nelson Laboratories, Kaye Markel has done it all. After more than 38 years of medical device quality control/assurance experience, Kaye Markel is retiring. Her career in the medical device field started when she enrolled at the LDS Hospital School of Nursing. It was there that she met her husband of 48 years. Kaye has been with Nelson Laboratories for the past 16 years acting as QA, Total Quality, and Regulatory Manager and Senior Inspector

Nelson staff have at times had fun at the expense

of Kaye. From practical jokes to her nickname "mad dog," Kaye has given many Nelson employees a good laugh. Kaye will miss the daily relationships she has formed with the staff, especially the family-like bond she has developed with the Nelson family. Long time co-worker Scott Dimond sums it up best, "Kaye always does her job well and will be greatly missed." She now plans on the trip of a life time, to Scotland, after which she'll return to Utah and donate her time to the local schools by helping children to read. The Nelson Laboratories staff want to wish Kaye the best of luck with her future endeavors.



Kaye Markel

MEDICAL DEVICE – An area of emphasis in the AAMI arena continues to be the harmonization of the major sterilization documents with the European Union. Since most of the current standards were already AAMI and ISO documents, the main chore involves harmonization with the current EN documents. These harmonization efforts have been ongoing for several years for the two main sterilization methods, Ethylene Oxide and Radiation, and are expected to be completed by late 2005 or in 2006. In addition, changes to allowable ethylene oxide residual levels are being addressed. Lastly, many working groups are currently working on new standards and guidelines for reusable medical devices. For more information on AAMI developments contact:

Martell Winters – Radiation questions – mwinters@nelsonlabs.com

Dan Floyd – EO questions – dfloyd@nelsonlabs.com

Emily Mitzel – Reusable devices questions – emitzel@nelsonlabs.com.

PHARMACEUTICAL – In a recent article published in the PDA Journal of Pharmaceutical Science and Technology volume 57, No. 6, Nov-Dec 2003, issues about barrier properties of vials that are stoppered but uncapped were addressed. The article titled "A method for Demonstrating Appropriate Environmental Protection for Capping Aseptically Filled and Plugged Vials" by John F. Arnold and Jeffery M. Price, presents concerns in the industry about these stoppered vials and medical device primary packaging. Several studies are being conducted to evaluate this concern using microbial aerosol challenge testing. For more information on this topic or to evaluate your package against a microbial aerosol to test barrier properties, please contact Sean Shepherd (sshepherd@nelsonlabs.com).

NUTRACEUTICAL – The Good Manufacturing Practice (GMPs) for Dietary Supplements – 21 CFR Parts 111 and 112 were expected to be published late spring. The implementation of the proposed rule has been delayed, due to concerns from industry associations about the impact of this regulation. It is expected that the regulation will be published in late 2004. Many companies are currently implementing appropriate quality systems to comply with the regulation. If you need assistance in establishing environmental monitoring, product or process quality control testing in anticipation of GMP compliance, please contact Amy Karren (akarren@nelsonlabs.com). A copy of the draft document and comments can be found at www.fda.gov or www.nnfa.org.

TISSUE BANKS AND PROCESSORS – As the FDA continues to work on the Good Tissue Practices (GTPs) the American Association of Tissue Banks (AATB) prepares to provide guidance to the industry. The AATB has organized a number of groups to address each critical part of the GTPs in a question and answer format. Each group will submit a series of questions and answers to AATB and FDA for approval as official guidance to the GTPs. It is hoped that this guidance will assist both FDA and tissue banks in heading down the same path as the GTPs become reality and begin to be enforced. For more information contact Martell Winters (mwinters@nelsonlabs.com).

INDUSTRY NEWS



Are you in control?

MICROBIAL ASSESSMENT OF THE MANUFACTURING AREA

By
Darcy Rawson, B.S.



During manufacturing, how can you be certain that your production area is free of microbiological contaminants that may affect your final product? Are you able to answer the above question — **“are you in control”** — affirmatively?

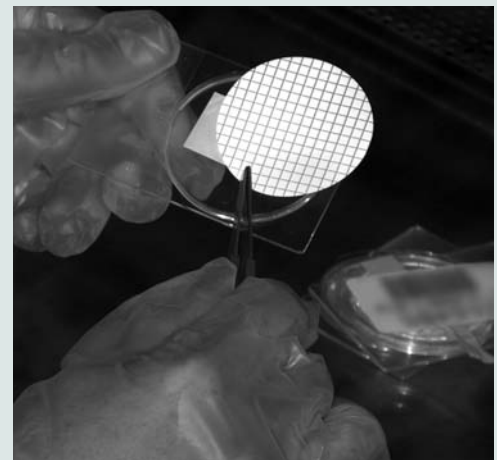
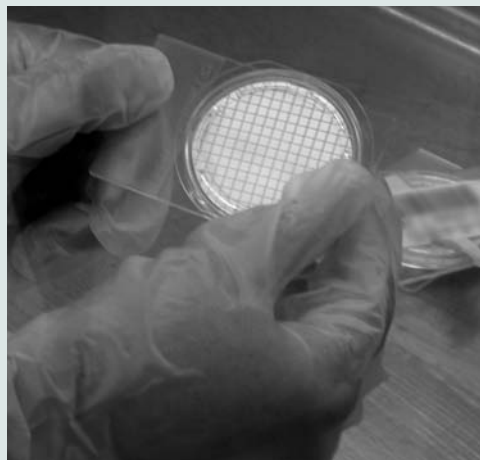
Upon completion of finished product testing, if test results are positive, will you know where the contamination came from? There are many possible sources of contamination. Inappropriately cleaned machines or instruments in the manufacturing area, poor air filtration, raw material and personnel are a few possible sources. If the source of contamination is not known, how can the problem in the manufacturing area be remedied?

ENVIRONMENTAL MONITORING (EM) CAN HELP FIND ANSWERS TO THESE QUESTIONS.

An EM program serves to demonstrate that facilities, people, and processes used in the manufacturing of products provide an environment that is reliable and consistently capable of maintaining acceptable microbial levels. Acceptable levels demonstrate that microbial contamination will not negatively affect the final packaged product. By implementing documented procedures and methods for monitoring microorganisms in the manufacturing area, this level can be demonstrated and trended.

Sites such as room air, compressed air, water, surfaces, and personnel should be considered in the monitoring process. These areas generate the greatest opportunity for contributing to product contamination. There are many methods and devices available for monitoring a manufacturing area. The most common methods are active air monitoring, passive air monitoring and surface monitoring.

Active air monitoring measures the number of colony forming units per unit of air. This is a quantitative method for monitoring viable organisms in the air. The two most common devices used to collect active air samples are impact samplers and filtration samplers. Generally, the air is actively pulled from the environment using a vacuum pump. With an impact sampler, the air sample directly impacts a nutrient agar medium. In filtration, the air is pulled over a filter and then the filter is aseptically placed on a nutrient agar medium. In both cases the agar medium

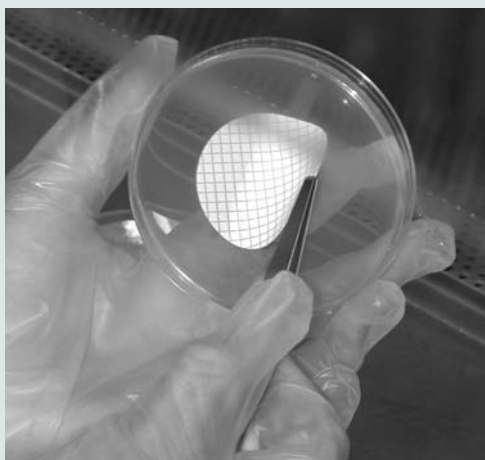


is then incubated. Any microscopic organisms that were present in the air at the time of sampling will multiply during incubation and form masses of cells visible to the naked eye known as colony forming units (CFU). Each colony detected by the analyst is counted as one colony forming unit. This gives an assessment of microbial contamination at the time of sampling. Compressed air systems can be monitored using a method similar to active air monitoring.

Passive air monitoring is a method for monitoring viable organisms falling onto work surfaces and products. In this method, a petri dish of nutrient agar or a filter is passively exposed to environmental conditions. As with active air monitoring, if filters are used they are aseptically placed on nutrient agar. This is demonstrated in the pictures below. The agar is then incubated and colony forming units are counted. Possible contamination can be calculated by determining the number of colony forming units per time period.

Surface monitoring is a method for monitoring viable organisms found on manufacturing surfaces. The most commonly used devices in surface sampling are contact plates and swabs. Convex contact plates or slides, filled with nutrient agar, are pressed directly on the sampling site. The agar is then incubated and colony forming units are counted. Swabs are swept over the sampling surface and then placed in a rinsing fluid and agitated. The rinse liquid is then assayed by filtering the liquid and placing the filter onto a nutrient agar. Again, the agar is incubated and colony forming units are counted. Contact plates are commonly used for flat surfaces such as work benches, floors and walls. Contact plates can also be used to monitor manufacturing personnel by pressing the agar onto an individual's uniform, lab coat or hands. Swabs are most commonly used for irregular surfaces that cannot be sampled with contact plates, such as pipes and tubing.

Environmental monitoring can be a very useful tool when performed and documented correctly. We recommend performing bacterial identification tests on environmental isolates in order to determine the bacterial flora of the production environment. An EM program can help to establish a baseline for decision making, demonstrate control of the manufacturing area and help detect an adverse trend that may potentially harm your product. An EM program should be manageable, meaningful and defensible so that you are able to say without question, "I am in control."



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FUTURE EVENTS BY NELSON LABORATORIES

THE SCIENCE OF STERILIZATION VALIDATION SEMINAR

TOPICS/SESSIONS:

STERILIZATION BASICS – *Dr. Jerry R. Nelson*
MATERIALS CHARACTERIZATION – *Karl
Hemmerich from STERIS*
BIOCOMPATIBILITY – *Kirk Poulsen*
RADIATION STERILIZATION – *Martell Winters*

ETHYLENE OXIDE STERILIZATION – *Dan Floyd*
STEAM STERILIZATION – *Emily Mitzel*
PACKAGING TESTING – *Gordon Ely*
PROCESS MONITORING – *Amy Karren*

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