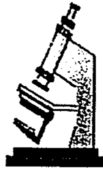
PMF

Pharmaceutical Microbiology Forum



PMF NEWSLETTER

A Publication of the Pharmaceutical Microbiology Forum
DISTRIBUTED INTERNATIONALLY

PURPOSE: To provide a forum for discussion of microbiology issues in the pharmaceutical and related industry. The information contained in this newsletter is the professional opinions of our members and does not represent the policies or operations of any corporation or government agency to which members may be associated. *PMF Newsletter* is intended to serve as an open forum and confidentiality will be maintained. The information in *PMF Newsletter* is solely for information purposes and is developed from sources believed to be reliable. Statements expressed constitute current opinions derived through analysis of available information and professional networking. Articles or opinions are for information only for PMF members to stimulate discussion and are not the views of the PMF board or regulatory agencies. The *PMF Newsletter* cannot make any representations as to the accuracy or completeness of the information presented and the publisher cannot be held liable for errors.

Volume 10, Number 1 February - March 2003

Inside this issue:

President's Message	1
Development of Precise Quality Control	2-4
How Well Do You Know Your History	4
USP Corner	5
Current Compendia	5
Alert Corner	5
History of Microbiology Answer Key	5
PMF Thread	6
Internet Addresses	6
Fungal ID Tips	7
Article Review	7

[illegible] Microbiology Forum
PMF 2003-2004 Organizational Board
[illegible] Diosynth RTP, Inc.
[illegible]@diosynth-rtp.com
[illegible] Amgen
[illegible]@amgen.com
[illegible] Valdes-Mora, Elite Microsource
[illegible]@aol.com
Secretary: Diane Hunhoff, Aventis Inc
Diane.Hunhoff@aventis.com
Assist. Secretary: Rich Almond
King Pharmaceuticals
[illegible]
Newsletter Editor: Ziva Abraham, Microrite, Inc.
[illegible]

Presidents Message

The 26th Revision of the United States Pharmacopeia (USP 26) and the 21st Edition of the National Formulary (NF 21) became effective on January 1, 2003. As I expected, Chapter <61> Microbial Limit Tests did not change and I now wonder when, if ever, the harmonization efforts will become a reality. Since 1998 (USP Open Conference on Microbiology for the 21st Century) "opportunities" to harmonize the microbial limit tests with the European and Japanese Pharmacopeias have been explored. In May of 2002, at the PDA/USP Joint Conference, this issue was again addressed. There was no consensus for some of the topics discussed. For example, there was no agreement on incubation times for total aerobic and total fungal count tests and on whether all batches of prepared media should be subject to growth promotion testing and which organisms should be used. However, the meeting attendees seemed to agree on the following issues:

- If the expected bioburden is low, one recovery medium will suffice.
- For validation of microbial recovery, minimum of 50 to 70% recovery from control is acceptable.
- Test for enterobacteria should only be applied to products from plant or animal source or liquid oral products.
- No need for routine anaerobic testing except for special products or conditions.

The group also agreed on a very important issue supported by current regulatory expectations: *objectionable organisms should not be specified in the pharmacopeias*. It is the manufacturers' responsibility to establish what microorganisms are objectionable and to ensure the test methods used will detect them. With this in mind, I ponder on additional roadblocks we might encounter on the path to global harmonization of the microbial limit tests. When dealing with environmental isolates we sometimes need to modify our standard methods to be able to achieve a particular inoculum level or obtain good recovery.

And talking about challenge inocula, this issue of the PMF features a unique method of standardization of microbial suspensions that can be used with great accuracy in challenge studies. We are all very familiar with the variability encountered in microbiological assays and challenge tests. It is nice to be able to gain accuracy in at least some of the variables and method steps, which leads me to the last subject I would like to bring to your attention: USP Chapter <1223> Validation of Alternative Microbiological Methods. The latest draft of this chapter, published in the PF-29, No.1, January-February 2003, addresses the need to apply principles of specificity, limit of detection, accuracy and precision when conducting microbial method validations. I strongly encourage our readers to review this document and give us your feedback in the PMF List (e-mail discussion group). More details on this chapter will follow in the next PMF issue.

Enjoy your reading!

Lucia Clontz

PMF Newsletter is published quarterly by the Pharmaceutical Microbiology Forum. Copyright ©1993 by the Pharmaceutical Microbiology Forum, 3166 Wood Valley Road, Panama City, FL 32405. Telephone (850) 763-5453. Send all inquires, letters, and comments to the above address, c/o L. Valdes-Mora. All rights reserved.

Development of Precise Quality Control Reference Material Standards for Microbiology

By Charlotte Morgan

From recent discussions on the PMF discussion group, it seems evident that the method and necessity of quality control (QC) for growth media for microbiological methods such as sterility tests and microbial limit tests are in doubt. Should media QC be quantitative? Is a presence/absence test sufficient? Does our media allow recovery in excess of 70%? Are our QC methods sufficiently precise to enable reliable and reproducible quantification of microbial recovery results? These are all questions that jump to mind when microbiological quality control is discussed. Obviously if a presence or absence result is all that is required, then no quantification is necessary, but is this method of testing not a little antiquated? If a test is worth performing, then the result should be quantifiable. However, any quantitative test is meaningless without sufficient controls to measure how well the method is performing. This brings another question to mind, "is a test quantitative if you are inoculating with a microbial suspension that has only been roughly enumerated?" In my mind the error lies with what you are using as your inoculant and how that inoculant is enumerated. The only reason why microbiology QC cannot be approached in the same manner used by analytical chemistry is that the reference controls currently used are inaccurate.

The accuracy and precision of a method can only be assured with adequate quality control. With the ever increasing demand for precise results for the enforcement of regulations, it is becoming clear that the quality controls of standard microbiological methodologies are not sufficiently adequate or accurate to ensure that standards are being adhered to. How can limits and acceptance criteria be set, when variation in the number of organisms within positive control samples can vary by 50%? At present, standard controls for microbiological testing still rely on the production of homogeneous dilutions of microbial cell suspensions, and enumeration by culturing techniques. This process results in a high degree of variation between samples, and is not accurately reproducible. The lack of consistency does not allow trends to be observed or comparisons to be drawn between or within laboratories. The only way to avoid this batch-to-batch variation is to change the fundamental thinking behind the preparation of microbial standards. Dilution methods produce error and large variations, therefore the necessary degree of accuracy required for precise reference materials cannot rely on manual diluting of microbial suspensions.

So how can this new generation of standard reference materials be produced? A technology is needed that can identify a desired population of microbes, consistently count a single cell, sort and dispense that single cell population and then preserve that quantum of microbes in a stable format for distribution. The novel difference between this approach to reference material production is that the quantity of micro-organisms are pre-determined and selected, therefore the number of micro-organisms will be standardised between batches and the variation will be consistently lower than the variation produced by dilution methodologies.

The newly developed bioball technology will produce a quality control containing a pre-determined 30 micro-organisms with a standard deviation equal or less than two. The process to produce these reference material standards initially involves culturing the required microbe under specific conditions to produce a population that is viable but has increased resistance to desiccation, and lyophilization. Once the culture is optimised for preservation, the microbes are analysed using flow cytometry and sorted into a single droplet, which is frozen and freeze dried to form a freeze dried ball.

Flow cytometry involves measuring physical or chemical parameters by passing a single file of cells through a core stream of fluid into the pathway of a laser beam. The laser light is absorbed, refracted or reflected by the cell, sending out scattered photons in different directions, which are detected on photodiode and photomultiplier tube (PMT) sensors that change the detected photons into an electrical signal. The sensors indicate the amount of light scatter that occurs in the forward direction (at a small angle from the original beam), which gives an indication of cell size, and also in a sideways direction (at large angles from the original beam), which indicates internal granularity and surface roughness. A second parameter used for cell differentiation is fluorescence, whereby cells are labelled with a fluorescent probe that absorbs light photons, and then emits photons at a longer wavelength, which is termed as fluorescence. The wavelength of the fluorescence emitted depends on the laser beam wavelength used to excite the fluorescent probes, and the type of fluorescent stain.

Figure 1: A typical plot from a flow cytometer analysis

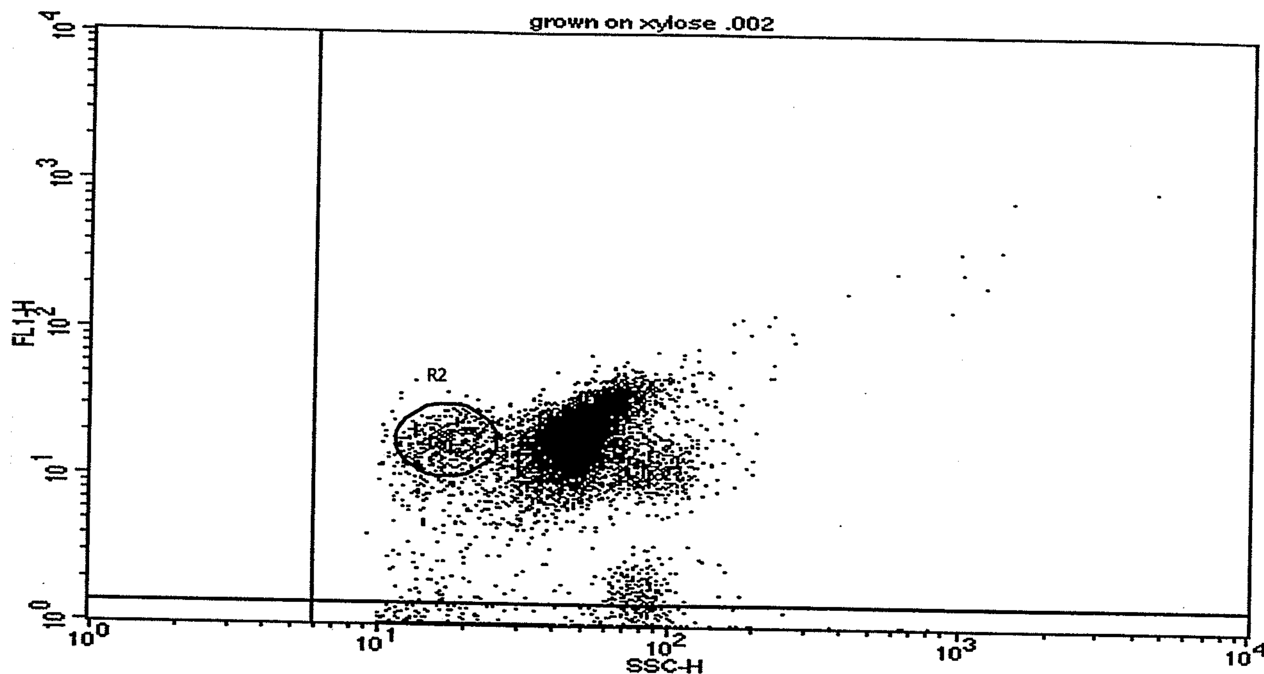
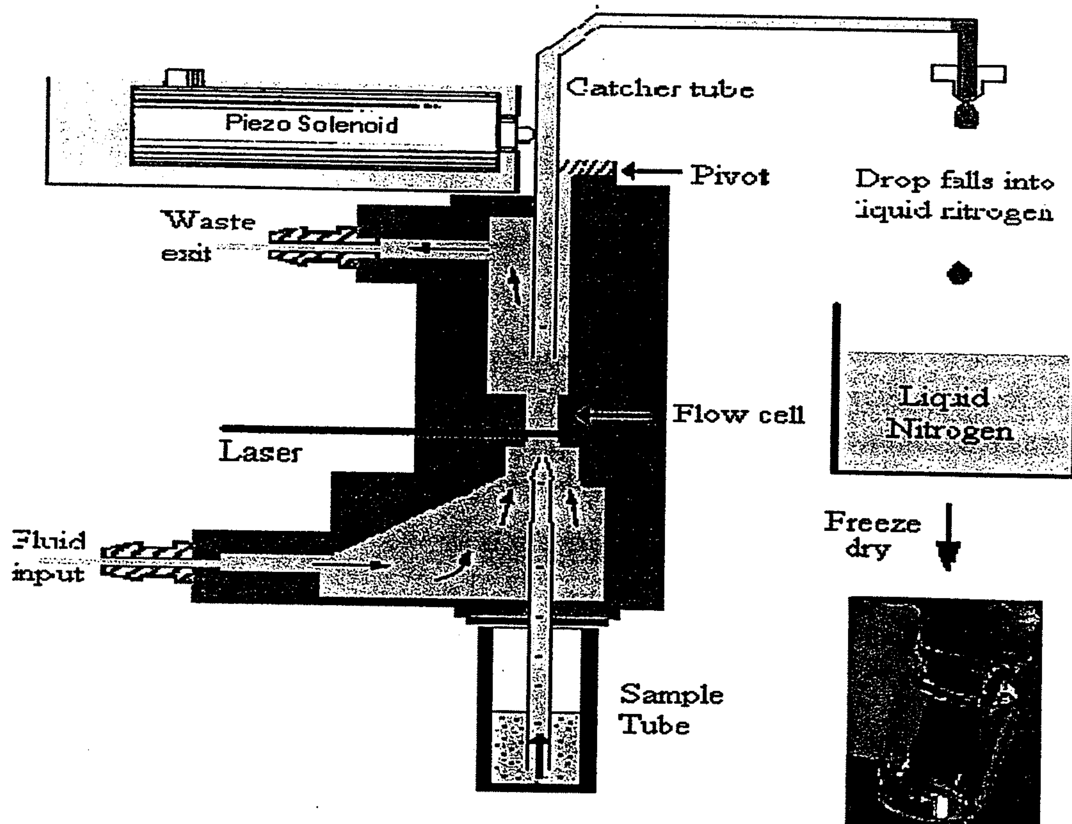


Figure 2: Schematic diagram of the bioball production technology



The flow cytometer uses the detection of forward light scatter, side light scatter and fluorescence at various wavelengths to identify a viable population with the use of various staining techniques. This identified population (refer to Figure 1), can then be sorted by selecting a sort region to include the population of interest (R2 population) and using a mechanical sorter arm that collects the identified cells from the continuous fluid stream, and separates them from the remaining contents of the sample. The sorting mechanism is outlined in Figure 2, the catcher tube is pivoted in and out of the stream of cells by a solenoid piston. The piston is activated by the detection of the cells within the required sort region and the catcher tube is timed exactly to move into position to collect that identified cell. The catcher arm can collect and sort up to 300 events per second. This sorted population is directed down a sort line to a dispensing tip, which is calibrated to form a droplet containing 30 counted organisms. This droplet is then collected in a glass vial that contains liquid nitrogen. The droplet is snap frozen and then is lyophilised (freeze dried). The freeze drying process sublimes the frozen water within the bioball into water vapour, which then travels along a pressure gradient (created by a vacuum pump) to a condenser, leaving a dry solid product, which is sealed under vacuum within the vial, and is ready for long term

A microbiology reference material in the format of a freeze dried ball can be added to a sample or to an agar plate without any losses of the micro-organisms. The ball is simply tipped into a sample or onto a plate and then rehydrated. No micro-organisms are lost in the vial or within a transfer pipette.

Future applications for this technology are not limited to bacteria. The same concepts can be applied to provide exact quantities of gene sequences, viruses, mammalian and plant cells for both research and clinical applications.

Further information on this technology can be obtained by contacting BTF on +612 8877 9150 or email at contact@biotechfrontiers.com.

About The Author

Charlotte Morgan is a research scientist with BTF – Biotechnology Frontiers, and is focused on the development of precise microbiology reference materials. Prior to joining BTF, Charlotte was a research scientist with Thames Water Utilities Ltd in the UK where she developed methods for the detection of *Cryptosporidium* and *Giardia* in water.

How Well Do You Know Your History of Microbiology?

Match the following

1	Gave the first accurate account of the visualization of bacteria and yeasts	Avery, MacLeod, and McCarty
2	Disproved spontaneous generation	Beijerinck
3	Introduced vaccination against smallpox with cowpox virus	Chamberland
4	Proved cholera is a water-borne illness	Cohn
5	Established the basis of a systematic classification of bacteria	Fleming
6	Set forth criteria (postulates) for ascertaining the causative agents of infectious diseases	Jenner
7	Conclusively proved that microbes are associated with dust particles in air	Koch
8	Introduced the practice of antiseptic surgery	Leeuwenhoek
9	Perfected the autoclave and introduced the bacterial filter	Lister
10	Introduced the diphtheria toxin skin test for measuring immunity to diphtheria	Pasteur
11	Discovered penicillin	Schick
12	Discovered genetic transformation of bacteria by DNA	Snow
13	Purified and determined the structure of endotoxin	Tyndall
14	Discovered filterable viruses (tobacco mosaic virus)	Westphal and Associates

Answer Key:

1. Leeuwenhoek
2. Pasteur
3. Jenner
4. Snow
5. Cohn
6. Koch
7. Tyndall
8. Lister
9. Chamberland
10. Schick
11. Fleming
12. Avery, MacLeod, and McCarty
13. Westphal and Associates
14. Beijerinck

Come Visit Our Website at http://www.microbiol.org/PMF.htm Are you aware of our on-line discussion group? Membership is FREE. To join, send an e-mail to Listserv@microbiol.org. Write ['Subscribe PMFlist' Firstname Lastname] as the first line of text. (message). You can ask, answer, or read questions and comments from your colleagues.

USP Corner

The PMF recommends that you *write directly to the USP with your comments on all proposals*. You can write representing your company, or as an individual scientist.

Any questions concerning USP documents should be sent to David Porter, Ph.D. You can reach Dr. Porter at: (706) 353-4514, via mail at United States Pharmacopeia, 126 Twinbrook Parkway, Rockville, MD 20852 or via e-mail at DAP@USP.org. When communicating with Dr. Porter, let him know you are a PMF member.

Current Compendia

US Pharmacopeia (USP) 26/National Formulary 21
First Supplement

European Pharmacopoeia (EP) 4
Supplement 4.4 April 2003
Supplement 4.5 July 2003

Japanese Pharmacopoeia (JP) XIV 2001

Chinese Pharmacopoeia (1995)

* If you use any other compendia, let us know for inclusion in this corner.

Warning Letters and 483s (issued in 2002)

"Failure to establish and follow appropriate written procedures... designed to prevent during manufacturing the introduction of objectionable organisms in drug products that are not required to be sterile in violation of 21 CFR 211.113 (a). You stated you based the release of these drug products on the antimicrobial preservative efficacy. However, a company's reliance on the antimicrobial preservative to reduce out of specification levels of microbes to within specification levels does not mitigate the requirement to produce products which meet specifications in the first instant."

"Failure to avert contamination in separate or defined areas designed to prevent contamination from occurring during manufacturing and processing operations [21 CFR 211.42 (c) (5)]. For example: The controlling and monitoring of movement of personnel, including maintenance personnel, components and ancillary materials such as tools necessary for equipment repairs/ adjustments into the filling area is not being performed."

"Failure to clean and maintain equipment and utensils at appropriate intervals to prevent contamination that would alter the safety, identity, strength, quality or purity of the drug product (21 CFR 211.67 (a). Specifically, you have failed to adequately complete the validation studies for current cleaning procedures specified for the PK Blender, and tabulating equipment utilized for production of human drugs."

"Failure to establish and follow adequate procedures to show that each lot of a component (purified water) that is liable to microbiological contamination that is objectionable in view of its intended use, is subjected to adequate microbiological tests before use [21 CFR 211.84(d)(6)]. Specifically, our investigators observed that the purified water system that is used for manufacturing sterile ophthalmic drugs is not adequately controlled or monitored. Your firm failed to investigate high microbiological counts on samples collected on 1/4/02. In addition, on 1/6/02, samples were collected from two locations not specified in procedures as sample locations, reportedly because the bacterial counts from two point of use outlets were "still high after sanitization".

PMF THREAD - Antimicrobial Effectiveness Testing

Question

We have received in our lab samples of cream and ointment to be tested for Antimicrobial Effectiveness. How can we treat these types of samples in order to have a good mixture of organism and sample? In addition, can anyone tell me about a reference document for Antimicrobial Effectiveness Testing?

Answer 1

Check USP and EP for the AET general chapters. We usually warm the ointment at 45°C and inoculate it. Then thoroughly mix it with a sterile spatula when needed.

Answer 2

The United States Pharmacopoeia and the European Pharmacopoeia are good reference materials. In our lab, we used to place the ointment in a 30-35°C incubator briefly before inoculation - in order to soften the ointment (plus, same just before each sampling). This method does not work as well with gels (they can be mixed using a syringe or pipette). Also, we used 1mL syringes for sampling instead of pipettes.

Answer 3

I would not treat the samples, but simply use thorough stirring with a small sterile spatula or tongue depressor. This has worked well for the creams I have tested. Even though the organism suspensions are not miscible with ointment, it is my experience that they can still be mixed so that they are evenly distributed throughout the sample. This requires much more time than testing aqueous solutions.

Answer 4

For reference documents you can use the pharmacopoeias (PhEur and USP), they both present test methods for Antimicrobial Effectiveness Testing (Preservative Efficacy Testing). The ointment can be spiked and mixed thoroughly with a sterile spoon. After this the desired volume can be weighted and diluted 1:10 with an appropriate diluting medium (i. e. Buffered Saline) and isopropylmyristate (IPM) to accelerate the solvation of the ointment. Another method could be an enzymatic breakdown and filter the sample through a 0.45-um filter.

Answer 5

You may need glass Petri dishes if you are using IPM as a diluent.

Answer 6

Some References:

Bloomfield, S.F. (1995) "Reproducibility and Predictivity of Disinfection and Biocide Tests", In: Microbiological Quality Assurance: A Guideline Towards Relevance and Reproducibility of Inocula, Brown, M.R.W. and P. Gilbert (eds.), Chapter 3.4, pp. 189- 220.

Leak, R.E. and R. Leech (1988) "Challenge Tests and Predictive Ability", In: Microbial Quality Assurance in Pharmaceuticals, Cosmetics and Toiletries, Bloomfield, S.F. et al. (eds.), Chapter 10, pp. 129-146.

Davison, A.L. (1988) "Preservative Efficacy Testing of Non-Sterile Pharmaceuticals, Cosmetics and Toiletries and Its Limitations", In: Microbial Quality Assurance in Pharmaceuticals, Cosmetics and Toiletries, Bloomfield, S. F. et al. (eds.), Chapter 9, pp. 120-128.

Internet Address	Description
http://www.fda.gov/bbs/topics/News/2002/new00829.html	FDA New Initiative
http://www.fda.gov/cder/dmpq/aseptic-cp.pdf	Sterile Drug Products Produced by Aseptic Processing Draft/ 09/27/02)
http://labcompliance.com/part11/durable-media.htm	Part 11 Information
http://www.microtestlabs.com/bioburdentesting.htm	Bioburden testing guidance

Fungal ID TIPS

Rhizopus

1. Hyphae scarcely septate.
2. Rhizoids and stolons present.
3. Sporangiophores brown, in tufts on stolons diverging from the point at which the rhizoids form.
4. Columella and sporangiospores seen in picture after the sporangia have burst.

IMPORTANT ANNOUNCEMENT

PMF Newsletter will be distributed electronically with the upcoming issue. All members need to provide their current e-mail address to Laura Valdes-Mora at Emsource@aol.com. Please mention PMF member e-mail in the subject line.

Article Review

Development of the Antimicrobial Effectiveness Test as USP Chapter <51>

Scott Sutton and David Porter
PDA Journal of Pharmaceutical Science and Technology, Volume 56, Number 6, November/December 2002

The authors in this article have reviewed the history, function and the technique of the Antimicrobial Effectiveness Test (AET) over a time period. This test first appeared in USP General Chapter in the 18th Revision in 1970 as "Antimicrobial Agents-Effectiveness." It was originally introduced as a test to demonstrate, in parenteral and ophthalmic products, the level of any added antimicrobial agent(s), the presence of which was declared on the label of the product concerned.

The test was redefined in USP XIX and the title was changed to "Antimicrobial Preservatives-Effectiveness" to prevent confusion about the chapter's impact on antibiotic test methods. In this revision, it was clarified that antimicrobials should not be used to reduce viable counts in lieu of Good Manufacturing Practices, and the toxicity of antimicrobials was addressed.

There were no major changes made to this chapter over a 15-year period, from 1975 to 1990, in USP XX, XXI, and XXII.

Since 1990, beginning with USP 23 through USP 25, several proposals were made with the goal of reducing interlaboratory variability of the test. The changes proposed during this period included the phenol coefficient test for qualifying stock cultures. The use of stock cultures within five passages from the original ATCC stock was the new requirement, storage hold times for prepared inocula and growth media were specified. In addition, product categories were renamed.

The international harmonization efforts for this test took place beginning with USP 23 through USP 25 . The authors have discussed in detail the additions and deletions that had to take place in USP, EP, and JP in lieu of the harmonization process. As of the latest editions of the USP, EP, and JP, the AET is still not fully harmonized.