PMF NEWSLETTER

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How Many?

The question of "*HOW MANY?*" is central to many tests in the QC microbiology laboratory. This question is commonly answered by some variant of the plate count method, but this method is not interpreted in the same fashion nor even performed in the same manner by all practitioners. This issue of the *PMF Newsletter* presents an examination of some of the relevant considerations.

Phil Geis contributes an interesting discussion on the purpose of preservation in the cosmetic industry. As non-sterile products with frequent and repeated use, cosmetic products offer real challenges to maintenance of product quality and stability.

Important Links:

Information on the PMFList at
http://www.microbiol.org/pmflist.htm

Past Issues of the *PMF Newsletter* at
http://www.microbiologyforum.org/news.htm

ATCC has launched a new service presented on page 4. The ATCC Standards Resource is a web-based searchable database for ATCC strains referenced in standards as well as QC strains suggested by vendors.

A Note on Sponsorships - The *PMF Newsletter* is provided as a free service to the bench microbiologist and those interested in the workings of the microbiology laboratory in an industrial setting. Free for use is not the same thing as free and PMF has to pay bills too. The PMF is dedicated to providing useful information available to as wide a group as possible, and charging subscriptions works against that goal. Therefore, like the PMFList, the *Newsletter* is accepting sponsorships as a way to generate operating capital. We acknowledge the first three sponsors in this issue - MIDI, Remel and Sartorius.

Scott Sutton, Ph.D.
scott.sutton@microbiol.org

The PMF Fall Forum is next month
You chose the topics!
http://www.highpeaks.us/

Counting Colonies

Scott Sutton, Ph.D.

Who Cares?

What is the fuss about in determining the number of colony forming units? After all, the CFU is only an estimate of the number of cells present. It is a skewed estimate at best as the only cells able to form colonies are those that can grow under the conditions of the test (incubation media, temperature, time, oxygen conditions, *etc*). Even among that group of microorganisms a colony does not represent a single cell, but rather cells that happened to be well separated on the plate and so can be distinguished after growth. A colony could arise from one cell, or several thousand. So why the fuss?

One reason for concern is that microbiology has a well-deserved reputation for being highly variable. Our lax attention to precision and accuracy in our measurements helps further this perception. We have allowed specifications for environmental monitoring, raw material bioburden, in-process bioburden and finished product bioburden to be imposed by regulation without regard for the ability of the method to support those specifications.

A second reason for concern is that now we are trying to introduce alternate microbiological methods into the lab. Being obsessive by training, we are trying to exceed measures of accuracy and precision in this exercise that the traditional methods cannot come close to matching. A good example of this is the Pharm Eur "Precision" requirement for an alternate method (quantification) to have a Relative Standard Deviation (RSD) in the range of 10-15% (1). While you might get lucky and hit this with dilutions whose plate counts are in the 150-250 CFU/plate range, - at lower plate counts this target value imposed by regulation will virtually guarantee a long, difficult and quite possibly unsuccessful, validation exercise.

Countable Range on a Plate

Literature

The general ranges in common acceptance for countable numbers of colonies on a plate are 30 – 300 and 25 – 250. The origin of those ranges is worth examination.

Breed and Dotterrer published a seminal paper on this topic in 1916 (2). They set out to determine the "limit in the number of colonies that may be allowed to grow on a plate without introducing serious errors…in connection with the proposed revisions of standard methods of milk analysis." They note that "the kind of bacteria in the material under examination will have an influence on the size of the colonies, and consequently, on the number that can develop on a plate." They also note that food supply can be an issue, that colonies close to each other on the plate may merge, and that neighbor colonies may inhibit growth or conversely stimulate growth. "Because of these and other difficulties certain plates in any series made from a given sample are more satisfactory for use in computing a total than are others. The matter of selecting plates to be used in computing a count becomes therefore a matter requiring considerable judgment."

Breed and Dotterrer chose their countable plates from triplicate platings of each dilution, requiring acceptable plates to be within 20% of the average. On this analysis, plates with more than 400 CFU were unsatisfactory, as were those of less than 30 CFU, with best results in the range of 50-200 CFU/plate.

The major paper from Tomasiewicz *et al* (3) provides an excellent review of the continued evolution of the appropriate number of CFU per plate from milk. They took data from colony counts of raw milk from three different experiments (each dilution plated in triplicate) and used to determine a mean-squared-error of the estimate for all plates. Their recommendation at the end of the study was for a countable range of 25-250 CFU/plate in triplicate. It is interesting to note that although the authors note that CFU follow a Poisson distribution, no mention is made of any data transformation used to approximate a normal distribution prior to the use of normal statistical analytical tools. Tomasiewicz *et al* provide excellent cautionary advice:

> "The data presented herein are not necessarily applicable to other systems. For automated equipment, the optimum range may well vary with the instrument… Furthermore, even if automation is not used appropriate numbers of colonies that should be on a countable plate can very widely, depending on many other variables. With soil fungi for example…"

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The compendia have recently harmonized a microbial enumeration test (4), and in this test recommend that the technician "Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 for TAMC and 50 for TYMC." In determination of the resistance of biological indicators, USP recommends a range of "20 to 300 colonies, but not less than 6" (5). However, the most complete description of the countable range is found in the informational chapter <1227> (6):

> "The accepted range for countable colonies on a standard agar plate is between 25 and 250 for most bacteria and *Candida albicans*. This range was established in the food industry for counting coliform bacteria in milk. The range is acceptable for compendial organisms, except for fungi. It is not optimal for counting all environmental monitoring isolates. The recommended range for *Aspergillus niger* is between 8 to 80 cfu per plate. The use of membrane filtration to recover challenge organisms, or the use of environmental isolates as challenge organisms in the antimicrobial effectiveness testing, requires validation of the countable range."

ASTM provides countable ranges of 20-80 CFU/membrane, 20-200 for spread plates and 30-300 for pour plates (7). The FDA Bacterial Analytical Manual (BAM) recommends 25-250 CFU/plate as a countable range (8).

Upper Limit

The upper limit of plate counts is dependent on a number of factors, as described previously. The major issues include the colony size and behavior (swarming?), and the surface area of the plate. The size particularly comes into play with plating a membrane for determination of CFU as the surface area of that membrane is so much smaller than that of a standard plate.

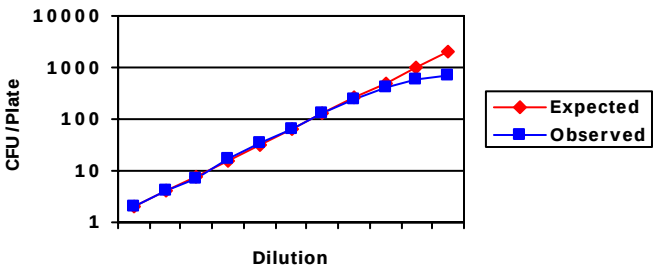

Figure 1 - Excessive CFU/plate

TNTC can be reported out several ways. ASTM (7) recommends reporting this out as >"upper limit". For example, a 1:10 dilution with more than 200 CFU on a spread plate would be reported as ">2,000 CFU/mL (or gram). FDA's BAM recommends counting the colonies from the dilution with plates giving counts closest to 250, counting a portion of the plate, estimating the total number and then using that number as the Estimated Aerobic Count. It is not clear to the author how this is greatly superior to guessing. In my opinion this is an invalid plating and needs to be done correctly at a later date (note I am strenuously avoiding the use of the word *retest*. This result invalidates the plating and therefore the test was not performed correctly.) I know this is a hardship to the lab, who were trying to reduce the plating load initially by not plating out suffi-

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cient dilutions. However, making a mistake initially is not a reasonable excuse to avoid doing it correctly after the mistake is recognized. If the lab wishes to use this "estimated count" it should, at a minimum, have it clearly described in their "counting CFU" SOP with a rationale as to when the plate counts are not critical and can be estimated in this fashion.

There are methods available if you should want to accurately determine the upper limit for a unique plating surface or a unique colony type. One is presented in the USP informational chapter <1227> (5) which is based on a pair-wise comparison of counts from a dilution series. This is based on the assumption that at the upper limit the observed numbers of CFU will fall off the expected numbers at some point (see Figure 1). This divergence will become significant at some point – that defines the upper limit of CFU/plate.

Lower Limit

A central concern in this determination is the reporting of the Limit of Quantification (which is what we are really interested in reporting) against the Limit of Detection (1 CFU). This is an important distinction if we are being held to specifications in the lower range.

ASTM recommendations focus on the LOD, and urge the user to report that answer out if no colonies are recovered (*ie* <10 CFU/mL for a 1:10 dilution) (7). If countable colonies are present, but below the countable range, count them anyway and report an estimated count.

USP (6) does not have a specific recommendation on how to report out these low numbers, but does note "Lower counting thresholds for the greatest dilution plating in series must be justified."

FDA BAM (8) recommends a different reporting format. In the FDA BAM method, all counts are recorded in the raw data, but the information is reported out as <LOQ. For example, a 1:100 dilution that yields counts of 18 and 12 would be reported out as <2,500. This is, in my opinion, the prudent course. The crux of the argument is that experiment studies have shown very poor accuracy in plate counts below 25 (see above). Theoretically we can argue that since the CFU follow the Poisson distribution, the error of the estimate is the square root of the average (USP <1227>). This leads to graphs such as in Figure 2 which shows us that as the CFU/plate drops below the countable range, the error as a percent of the mean increases rapidly. This confusion between the Limit of Detection and the Limit of Quantification for plate counts has led to some very difficult situations (as discussed below).

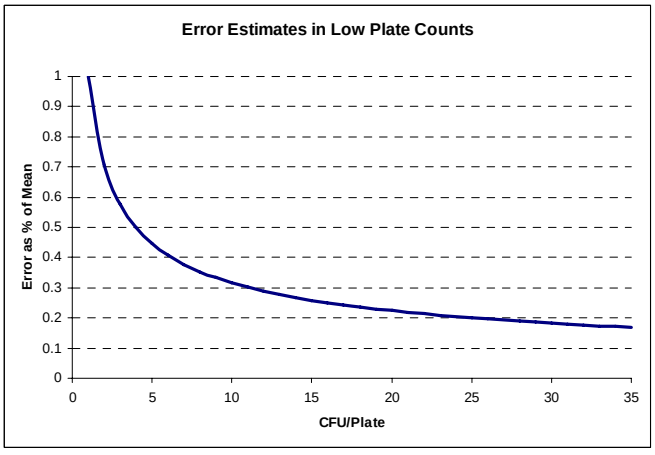

Figure 2 - Error at low CFU/plate

Unusual Situations

What About Two Dilutions with Countable Colonies?

Ideally you would never see two separate dilutions with counts in the countable range, as the countable ranges cover a ten-fold range of CFU. However, this *is* microbiology.

ASTM recommendations (7) urge you to take both dilutions into account, determining the CFU/mL (or gram)

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separately for each, then averaging the results for the final result. Breed and Dotterrer (2) also used several dilutions if the numbers fit the QC requirements (see below). FDA BAM has no recommendations in this situation.

While the argument can be made to use all counts, this is a stronger argument if triplicate plates are used and QC limits are in place to discard erroneous plates.

A strong argument can also be made to take the dilution providing the larger number of CFU in the countable range. This approach minimizes two concerns, that the errors in the estimates increase with increasing serial dilutions, and that the error in the estimate increases with decreasing plate counts. Use of the smaller dilution (*eg* 1:10 *vs* 1:100) could be justified from this perspective.

Whichever method used should be documented and justified in the "Counting CFU" SOP.

What about QC Limits on Replicate Plate Counts?

Periodically there are recommendations to establish Quality Control limits on replicate plate counts. Breed and Dotterrer in their 1916 paper (2) required valid plate counts from triplicate plates to provide estimates of CFU/mL within 20% of the mean. In other words, all plates were counted, each plate's CFU count was used to estimate the original CFU/mL, then each estimate was evaluated. If the individual plate's estimate was within 20% of the mean, it was deemed acceptable. This method is not practical in the QC lab.

Establishment of QC limits for plate counts works best if you have at least three replicate plates for each dilution. The average of the dilution replicates can be determined, variant counts (hopefully no more than one plate per triplicate plating) discarded and the final average determined. If you try this with duplicate plates you frequently end up with trying to average the results of one plate. While this method looks good on paper, the prudent lab manager will evaluate some historical data before instituting it as a practice.

The method used to QC individual plate counts, if used, should be documented and justified in SOP, along with the response to finding variant counts.

Can I plate 10 1 mL samples to plate a Total of One 10 mL Sample?

There have been suggestions that a larger volume of material may be plated across several plates, and the results reported out for the larger volume. For example, plating 10 1 mL samples on 10 different plates, and then reporting it as if a 10 mL sample was plated. This approach is flawed in that it ignores several sources of variability in plating including sampling error, plating errors, growth/incubation error and counting errors (9, 10). The correct interpretation for this situation that you have just plated 1 mL ten times, not 10 mL once. The numbers might be averaged, they cannot be added.

Rounding and Averaging

To discuss this we need to determine what the significant figures might be in the measure. For raw colony counts, common practice determines that the CFU observed determine the significant figure, and that the average is one decimal to the right of that number (sticklers for accuracy will report the geometric mean rather than the arithmetic mean given the Poisson distribution followed by CFU). In reporting, it is common practice to report out as scientific notation using two significant figures. This requires rounding.

USP (11) and ASTM (7) both round up at five if 5 is the number to the right of the last significant figure. FDA BAM has a more elaborate scheme, rounding up if the number is 6 or higher, down if 4 or lower. If the number is 5, BAM looks to the next number to the right and rounds up if it is odd, down if it is even.

This is one of those situations where you want everyone to do the calculations the same way (I am hard

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Internet Address	Description
http://www.siteadvisor.com/	McAfee SiteAdvisor - Free utility from McAfee that integrates into Google to provide a safety rating for google search returns. This utility warns you before you interact with a dangerous Web site.
http://www.scirus.com/	Scirus describes itself as the most comprehensive science-specific search engine on the Internet. Driven by the latest search engine technology, Scirus searches over 250 million science-specific Web pages to pinpoint scientific, scholarly, technical and medical data on the Web and find the latest reports, peer-reviewed articles, patents, pre prints and journals that other search engines miss.
If you have found an Internet site that contains information of relevance to pharmaceutical microbiology, please let us know.	

Purpose of Cosmetic Preservation

Phillip A. Geis, Ph.D.
Procter & Gamble

A recent article from Europe attempted to evaluate what the authors saw as recent trends in cosmetic microbiology (1). Largely focused on EU regulatory measures of the last few years, the authors added a brief discussion on the purpose of preservation that surprisingly (at least to this microbiologist) appeared to see its primary if not sole role as protection during manufacturing. This clarifies what I believe the cosmetics industry accepts – that the primary objective of cosmetic preservation is to protect against consumer contamination.

With the reported potential for serious, even fatal health consequences as well as more sublime compromise of product esthetics, microbiological contamination is a serious threat to cosmetic product quality. Most consumers are aware that mascaras and other cosmetics used around the eyes offer unique microbiological risks and their effective preservation is a special concern. What may not be so widely recognized is the importance of preservation for other cosmetic products. Consider shampoo – a category of cosmetics product sold in great volume globally and for which the most significant preservative-related database exists. A simple Google® search will find numerous recommendations that consumers dilute shampoos. This simple act of adding tap water dilutes and compromises preservative efficacy while inoculating with classic microbial contaminants such as *Pseudomonas aeruginosa* (2, 3). Even without dilution, preservative efficacy has clearly been correlated with microbiological success and failure in consumer use (4). Whereas contamination inevitable with poorly-preserved or diluted products may go unrecognized beyond esthetic changes in product viscosity, color or odor (5), it can offer real peril to consumer health. My late friend Roy Hopfer reported (6) a fatal infection that was traced directly to a shampoo contaminated by dilution. As the victim was undergoing chemotherapy for leukemia, one might consider the risk limited to an unique and relatively small group. However, consider that your loved ones, friends and coworkers at special risk - pregnant women, diabetics, cancer patients undergoing chemotherapy and organ transplant recipients, those with chronic diseases and the very old and very young compose greater than 20% of the US population (7). The more severely compromised are even at risk of indirect contact with contaminated materials where medical workers served as links between contaminated cosmetics in their homes and patients suffering serious, even fatal infections in the hospital setting (8, 9).

Nevertheless, preservation does play a role in manufacturing as the price of entry for success. Indeed manufacturing contamination is an important concern and a review of US FDA cosmetic records reveals that microbiological contamination is a significant cause of cosmetic product recalls, rare as are recalls for this category of regulated products (10). In the manufacturing plant, the role of preservatives is to establish a threshold of efficacy. These ingredients are not the primary means of microbial control as well-preserved products – even antimicrobial raw materials themselves (11, 12) – will suffer inevitable contamination if made on systems lacking microbiological control. By contrast, inadequately-preserved products may be successfully produced on a system under a high degree of hygienic control but risk failure in the hands of the consumer. As our industry knows, manufacturing hygiene is the primary determinant of as-made product quality.

The distinction is important as the cosmetics industry does focus on consumer protection as the primary purpose of preservation and on good manufacturing practices and hygiene as the primary means to protect manufacturing facilities. Failure to clarify can leave the mistaken impression that preservatives have no value to the consumer.

References

1. Orus, P., and S. Leronaz. 2005. Current trends in cosmetic microbiology. *Int. Microbiol.* 8:77-79.
2. Reuter, S., Sigge, A., Wiedeck, H., and Trautmann, M. 2002. Analysis of transmission of *Pseudomonas*

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ATCC Standards Resource™

Joseph B. Perrone, ScD
American Type Culture Collection

ATCC's newly launched Web-based standards search tool gives you a quick and easy way to look up which cultures are required for a specific standard, guideline or test method.

In May, ATCC® introduced a Web-based search tool that allows scientists and laboratory professionals to efficiently find out which ATCC-held strains are specified in a particular standard.

The Standards Resource cross-references ATCC cultures with a database of widely used standards from professional organizations, standards-setting bodies, government agencies and commercial companies. The database includes standards and test methods citing ATCC products developed by organizations including USP, AOAC International, ASTM International, CLSI (Clinical and Laboratory Standards Institute), FDA, British Pharmacopoeia and many others.

Also included in the database is information on ATCC strains recommended by commercial firms. For example, BD, bioMérieux, Dade Behring and Dupont Qualicon specify ATCC strains as controls for rapid identification, minimum inhibitory concentration, antibiotic susceptibility panels and other commercial assays.

Users of the ATCC Standards Resource have several search options. You can search by the name of the standard, the number of the standard, or by the name of the standard-setting organization. In addition, you can search by commercial manufacturer of testing instrumentation, commercial product (testing equipment) and by ATCC standard reference culture.

When you enter a specific standard, the Resource will return information about the ATCC reference materials necessary for carrying out that standard procedure. Alternatively, when you enter a particular ATCC standard reference culture, you can view all standards-related information associated with that material. You can also link directly to the homepage of the standard-setting organization to obtain a copy of the standard or to the ATCC item detail page to obtain additional culture and growth information.

The ATCC Standards Resource can be accessed via the ATCC home page http://www.atcc.org:

1. Click on the "**ATCC Standards**" button on the left-hand navigation bar to get to the Standards Resource page.
2. From there, click the "searchable database"

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aeruginosa between patients and tap water outlets. *Crit. Med. Care* 30: 2222-2228.

3. Chaidez, C., and C. Gerba. 2004. Comparison of the microbiologic quality of point-of-use (POU)-treated water and tap water. *Int. J. Environ. Health Res.* 14:253-260.
4. Brannan, D.K., J.C. Dille, and D.J. Kaufman. 1987. Correlation of in vitro challenge testing with consumer use testing of cosmetic products. *Appl. Env. Microbiol*. 53:1827-1832.
5. Geis, P.A. 1988. Preservation of cosmetics and household products: rationale and application. *Dev. Indust. Microbiol*. 29:305-315.
6. Fainstein, V., N. Andres, J. Umphrey, and R. Hopfer. 1988. Hair clipping: another hazard for granulocytopenic patients. *J. Infect. Dis*. 158:655-656.
7. Gerba, C.P., J.B. Rose and C.N. Haas. 1996. Sensitive populations – who is at greatest risk? *Int. J. Food Microbiol*. 30:113-23.
8. Passaro, D.J., J. Waing, L. Armstrong, F. Bolding, B. Bouvier, J. Rosenburg, A.W. Reingold, M. McQuitty, S.M. Philpott, W.R. Jarvis, S.B. Werner, L.S. Tompkins, and D.J. Vugia. 1997. Postoperative *Serratia marcescens* wound infections traced to an out-of-hospital source. *J. Infect. Dis*. 175:992-995.
9. Becks, V.E., and N. M. Lorenzoni. 1995. *Pseudomonas aeruginosa* outbreak in a neonatal intensive care unit: a possible link to contaminated hand lotion. *Am. J. Infect. Control* 23: 396-398.
10. Wong, S., D. Street, S.I. Delgado, and K.C. Klontz. 2000. Recalls of food and cosmetics due to microbial contamination reported to the US Food and Drug administration. *J. Fd. Protect*. 63:1113-1116.
11. Tejpratap S. P. Tiwari, B. Ray, K. C. Jost, M. K. Rathod, Y. Zhang, B. A. Brown-Elliott, K. Hendricks, and R. J. Wallace. 2003. Forty years of disinfectant failure: outbreak of postinjection *Mycobacterium abscessus* infection caused by contamination of benzalkonium chloride. *Clin. Infect. Dis.* 36:954–962.
12. Reiss I, A. Borkhardt, R. Fussle, A. Sziegoleit, and L. Gortner. 2000. Disinfectant contaminated with *Klebsiella oxytoca* as a source of sepsis in babies. *Lancet* 356:310.

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link or the “Search Standards Database” button to go to the **Standards Search page**.

The following are answers to some of the most frequently asked questions about the Standards Resource:

1. **What USP standards cite ATCC reference cultures and which cultures are required for each?**
   Use the Organization/Company dropdown to find and highlight "**U.S. Pharmacopoeia**". Press the "**Search**" button and the search results will provide a list of USP standards that cite ATCC cultures as well as the ATCC reference standards necessary to perform each standard method.

2. **Which ATCC reference cultures do I need to perform the USP standard method in General Chapters: <71> Sterility?**
   Search by standard number:
   Select “**Test Number**” from one of the Field Search dropdowns and type “71” in the associated text box, then select “**U.S. Pharmacopeia**” from the Organization/Company dropdown and press the "**Search**" button. The search results will provide the ATCC reference cultures necessary to perform USP standard method General Chapter <71>, Sterility.
   OR
   Search by standard name:
   Select “**Test Name**” from one of the Field Search dropdowns and type “Sterility” in the associated text box, then select “**U.S. Pharmacopeia**” from the Organization/Company dropdown and press the "**Search**" button. The search results* will provide the ATCC reference cultures necessary to perform USP standard method General Chapter <71>, Sterility. [*Search results also include General Chapters: <1211> Sterilization and sterility assurance of compendial articles.]

3. **Which ATCC reference strains do I need to perform QC testing of the bioMérieux “Vitek 2”?**

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Upcoming Events

September

- 28th - 29th **Investigating Microbial Contaminations**
  Location: Northbrook, IL
  Web Site: http://www.microrite.com/

October

- October 2nd - 3rd **New Chapters of the European Pharmacopoeia**
  Location: Strasbourg, France
  Website: http://www.pheur.org/site/page_647.php
- 12th - 13th **PMF Fall Forum**
  Location: Rochester, NY
  Website: http://www.highpeaks.us/2006/PMF.Fall.Forum/
- 25th CTFA Microbiology Seminar
  Location: Newark, NJ
  Website: www.ctfa.org
- 27th - 28th **PDA Microbiology Meeting**
  Location: Bethesda, MD
  Website: http://www.pda.org/microbiology2006/

November

- November 29th - 30th **15th Annual PharMIG Meeting**
  Location: Nottingham Belfry Hotel
  Contact: Maxine Moorey (maxine@pharmig.org.uk)

Offering In-House Courses on Microbiology/ Aseptic Processing:

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm
- USP
  Contact Steven Paul (stp@usp.org) for information on the course "Fundamentals of Microbiological Testing"

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.

The 2006 PMF Fall Forum is Coming!

The 2006 PMF Fall Forum will be October 12-13 in Rochester, New York. Come and discuss current topics with the leaders in the field—those who publish are the invited speakers!

Speakers and Topics include (to date):

- Culture Collections and Cryopreservation; Liz Kerrigan (ATCC)
- Harmonized Microbial Limits Tests; Scott Sutton (PMF)
- Environmental Monitoring Databases - Validation Concerns; Robert Ferer (Vectech)
- Analysis of EM Data; David Hussong (FDA - invited)
- Environmental Monitoring for non-steriles; Jeff One (Abbott)
- Discussion of Part 9000, Microbiology, in Standard Methods for the Examination of Water and Wastewater; Margo Hunt (EPA)
- Aseptic Process Simulations (media Fills) Program at a Parenterals Pharmaceutical Company; Roxanne Robles-Torres (Wyeth)
- What Is Disinfectant Validation? Jose Martinez (JEM Consulting)
- Microbiology Training Programs; Jackie Hewitt (Luitpold Pharmaceuticals)
- Training for Aseptic Processing Environments; Anne Dixon
- Biological Indicators; Jeanne Moldenhauer (Vectech)

Discussion List Update

PMFList:
Number of Subscribers: 1,679
Number of Countries: 63
Number of Messages Last Month: 221

PSDGList (Pharma Stability Discussion Group):
Number of Subscribers: 841
Number of Countries: 19

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

A sister Email is devoted to topics in the **stability testing** of pharmaceuticals, medical devices and personal products. To **join the PSDGList**, visit http://microbiol.org/psdglist.htm and register.

You can ask, answer, or read questions and comments from your colleagues. Archives of the lists are available at:

- http://lists.microbiol.org/archives/PMFLIST.html
- http://lists.microbiol.org/archives/PSDGLIST.html

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pressed to come up with a situation in a lab where you want everyone to do it differently). Be sure to include direction and its justification in the "Counting CFU" SOP if it does not already exist in a separate SOP.

Impact on Specifications and Environmental Monitoring Control Levels

We are back to the question of *WHO CARES?*

If you are faced with a finished product bioburden of NMT (Not More Than) 100 CFU/gram, and your method suitability study requires a 1:100 dilution of the product to overcome any antimicrobial effects, then how are you to test it? Common practice is to perform the 1:100 dilution, perform a pour plate of 1 mL in duplicate and if 2 colonies grow on either plate, the product fails specification. This common practice is scientifically unsupportable – it confuses the Limit of Detection with the Limit of Quantification for the plate count method.

Let's take a look at environmental monitoring alert and action levels for aseptically produced products. Hussong and Madsen (12) recently published a thoughtful review of this topic where they argue that the levels of acceptable CFU for many room classifications are below the noise level of plate count technology (*eg* in the range of 1-2 CFU/m^3). In addition, environmental data can be extremely variable, much more so than controlled lab studies as the numbers of microorganisms, the physiological state of the isolates, even the species are completely out of the control of the investigator. In addition the numbers do not conform to a normal distribution as there are sporadic counts with a count of "zero" CFU predominating. They conclude that since the numbers are unreliable, the trend in the data is the only important consideration, and that EM counts cannot be used for product release criteria. A separate treatment of this subject was presented by Farrington (13) who argues that the relationship between EM data and finished product quality is a widely held, but unproven belief, compounded by the problems in accuracy with the low counts generated by plate count methodology.

Conclusions

In conclusion, all methods have limitations. One of the major limitations to the plate count method is the relatively narrow countable range (generally considered to be 25-250 CFU bacteria on a standard petri dish). The currently prevailing confusion between the Limit of Detection (1 CFU) and Limit of Quantification (25 CFU) for the plate count method creates a larger degree of variability in microbiology data than is necessary. An unfortunate regulatory trend in recent years is to establish expectations (specifications, limits, levels) for data generated by the plate count method that the accuracy of the method cannot support. This is a real opportunity for modification of current practice to approach the goal of "science-based regulation".

References

1. Pharm Eur. 2006. 5.1.6 Alternative Methods for Control of Microbiological Quality. Pharm Eur vol.5.5 pp 4131-4142.

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Select "**bioMérieux**" from the Organization/ Company dropdown and "Vitek 2" from the Instrument Name dropdown list [or simply select Vitek 2]. Press the "**Search**" button and the search results will provide a list of all the Vitek 2 QC sets and the ATCC reference strains necessary to perform each test.

4. **How can I get a copy of USP standard method <71>, Sterility, or any of the standards listed?** You can obtain a copy of the standard method from USP. For your convenience, we have linked the name of the standards organization on the search results page to their home page. In this example click the link "**U.S.Pharmacopoeia**" to go to the USP homepage. All organizations listed have a link to their home page where you can inquire about the standard or any of their other products.

For additional information, contact: standards@atcc.org.

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2. Breed, R and WD Dotterrer. 1916. The Number of Colonies Allowable On Satisfactory Agar Plates. *J Bacteriol*. 1:321-331.
3. Tomasiewicz, D.M.et al. 1980. The Most Suitable Number of Colonies On Plates for Counting. *J Food Prot*. 43(4):282-286.
4. USP. 2006. <61> Microbial Examination of Nonsterile Products: Microbial Enumeration Tests. USP 29 Suppl 2. August 1, 2006 . United States Pharmacopeial Convention. pp. 3757-3759.
5. USP. 2006. <55> Biological Indicators – Resistance Performance Tests. USP 29. United States Pharmacopeial Convention. pp. 2501-2503.
6. USP. 2006. <1227> Validation of Microbial Recovery from Pharmacopeial Articles. USP 29. United States Pharmacopeial Convention. pp. 3053-3055.
7. ASTM. 1998. D5465-93(1998) Standard Practice for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods
8. FDA. 2001. Chapter 3 – Aerobic Plate Count *In* Bacteriological analytical Manual *Online* at http://www.cfsan.fda.gov/~ebam/bam-3.html#6-International
9. Jennison, MW and GP Wadsworth. 1940. Evaluation of the Errors Involved In Estimating Bacterial Numbers by the Plating Method. *J Bacteriol*. 39:389-397.
10. Weenk, G.H. 1992. Microbiological Assessment of Culture Media: Comparison and Statistical Evaluation of Methods. *Int J Food Microbiol*. 17:159-181.
11. USP. 2006.*General Notices:* Significant Figures and Tolerances. USP 29. United States Pharmacopeial Convention. p. 4.
12. Hussong, D and RE Madsen. 2004. Analysis of Environmental Microbiology Data From Cleanroom Samples. *Pharm Technol*. Aseptic Proc Issue: 10-15.
13. Farrington, JK. 2005. Environmental Monitoring in Pharmaceutical Manufacturing - A Product Risk Issue. *Amer Pharm Rev*. 8(4):26-30.

Press Release

Cold Filterable Media now available from Remel for Media Fill Trials

Oxoid Cold Filterable Media, now available in the U.S. from Remel, are designed to be the perfect solution for trouble-free media fill trials. Standard Tryptone Soya Broth often results in slow filtration or filter blockage when used during fill trials. Oxoid Cold Filterable Tryptone Soya Broth and Cold Filterable Vegetable Peptone Broth readily dissolve in cold water and are highly filterable at room temperature. Oxoid Cold Filterable Media are designed to flow freely through a 2 µm filter and are the first document high filterability with a lot-specific Vcap value. By allowing faster filtering (without the need to heat the media or change blocked filters), these products save valuable time in completing media fill trials and more accurately mimic the aseptic production process.

The Parenteral Drug Association recently published "*Guidance for Industry: Sterile Drug Products by Aseptic Processing – Current Good Manufacturing Practice*" which documents ramifications for many aspects of the design, operation and monitoring of aseptic production processes. These new pharmaceutical guidelines state that media fill trials should mimic the production process as closely as possible. This means that now, the media used in fill trials passes through the same aseptic filter trains as live product.

In addition to the benefits of high filterability at room temperature, Oxoid Cold Filterable Media are gamma-irradiated with a dosage validated as lethal to *Mycoplasma*, yeast, mold, and bacteria (including bacterial spores). Cold Filterable Vegetable Peptone Broth offers an alternative that is completely free of animal derived materials.

Cold Filterable Media are tested according to specifications for growth of microorganisms established by the U.S., European, British, and Japanese Pharmacopoeia standards.

Oxoid Cold Filterable Media are available from Remel Inc. (Lenexa, KS), a global provider of a wide range of high quality Microbiology products used by clinical, industrial, research, and academic laboratories. Remel provides quality products, excellent technical and customer support and convenient next-day delivery. For more information about Remel or its products, visit www.remel.com or call 800-255-6730.

FOR MORE INFORMATION CONTACT:
Kelly Fowler (913) 895-4234

Note from editor: This press release is presented in its entirety to make the product availability known. Similar products are available from several vendors, and the appropriate use of any product should be demonstrated on site. Publication of this press release, and others in the future, should not be interpreted as endorsement of any particular product or service by the PMF.

CTFA Science Week Microbiology Seminar

The Cosmetic, Toiletry, and Fragrance Association will hold its annual microbiology seminar, Resolving Microbiological Issues in Manufacturing, on October 25, 2006 at the Airport Marriott in Newark, New Jersey. Pharmaceutical microbiologists will find many of the topics covered at this seminar to be highly relevant to their own operations. Speakers at the CTFA Microbiology seminar will focus on practical measures to prevent contamination in manufacturing as well as techniques to identify and correct microbiological contamination problems in personal care products. Ron Fisher of NVE, Inc. will provide an overview of the Micro Investigation Process. Andrew Dick of Johnson & Johnson Consumer Products will discuss "Design, Cleaning and Sanitization" in manufacturing. Pat Palmieri (Process Optimization, Inc) will conclude the morning session with a presentation on Hygiene Practices and Training. The afternoon session will begin with Mary Connor of Del Labs speaking on Best Lab Practices and be followed by Dr. Scott Sutton on Preservative Neutralization. The seminar will conclude with Dr. Eric Brown of the FDA Center for Food Safety and Nutrition (CSSAN) who will speak about phenotypic and molecular methods to identify and trace microbial contaminants. Registration forms and additional information on Resolving Microbiological Issues in Manufacturing and other seminars on Cosmetic Ingredient Nomenclature, Good Manufacturing Practices, Color Technology, and Cosmetic Safety Risk Assessment during CTFA's Science Week can be found at www.ctfa.org or by contacting John Krowka at krowkaj@ctfa.org.