# PMF NEWSLETTER

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## An Issue with Some Regulatory Topics

This issue attempts to provide some guidance on the technique of seed lot culture and how to use it in a QC laboratory. The seed lot technique can be a very useful tool in meeting several QC objectives but persistent questions on the PMFList suggest that this is not a widely understood procedure.

In a departure from the normal type of article we also explore a recent warning letter issued by FDA. This warning letter is interesting in that it deals with the microbiology and the role of in-process tests on the quality of finished products, corrective actions to resolve investigations and the bacterial endotoxin test. The *PMF Newsletter* does not normally discuss regulatory topics or 483s, warning letters and such, but this one is worth spending some time discussing.

**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

A third section in this issue deals with some recent news items. Please let us know what you think of this offering—if people like it we will continue providing recent items from the news wires.

Finally, the PMF has a full conference schedule for next year. If you are interested in participating in leading in, or speaking at, a PMF conference, please let us know. We are open to all ideas and looking for the opportunity to provide new people a forum to speak and share their ideas.


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# Seed Lot Technique

**Scott Sutton, Ph.D.**

We frequently see the Seed Lot Technique referenced in the USP, literature publications, and conference presentations. I would like to take a few minutes to explore the technique in some detail.

The goals of the seed lot technique in our industries is three-fold:

1. To provide a method for generation of a working culture no more than 5 passages removed from the original stock from the national repository
2. To provide a method for generation of a pure culture for laboratory work
3. To provide a method for generation of a traceable culture for laboratory investigations

Let's look at each of these goals separately.

## No More than 5 Passages Removed

The goal of "no more than 5 passages removed" is now pretty common in the compendia, but not so commonly provided are the details on exactly how to accomplish this. This explanation will rely on Figure 1 below.

In this design, the lyophilized culture is received, resuscitated and grown in broth. This growth step is the first

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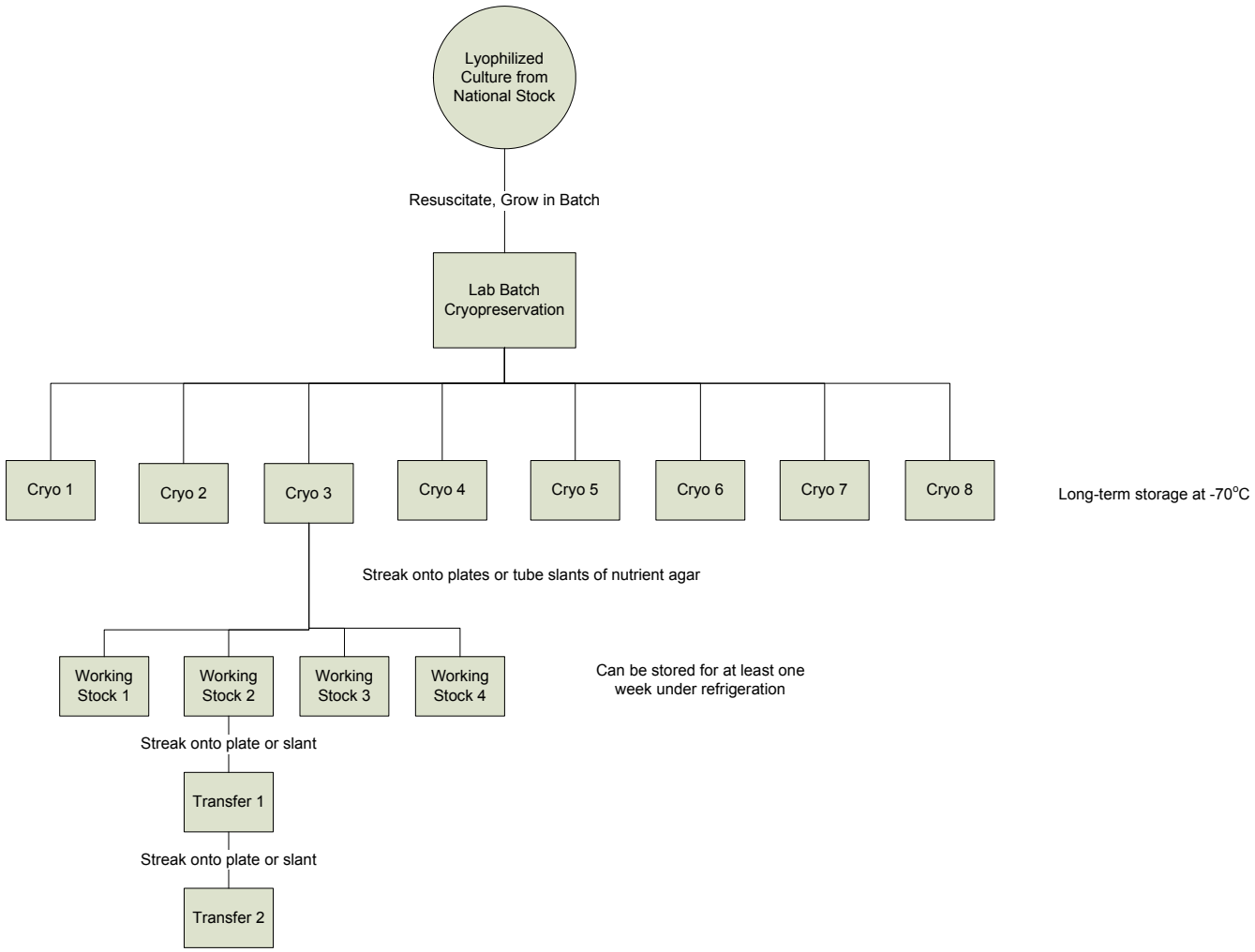
Figure 1. Typical Seed Lot Culture Design



# A Warning Letter About Process Controls

Scott Sutton, Ph.D.

The US FDA issued Ben Venue Laboratories a Warning Letter (Nov. 16, 2008) involving concerns about in-process test practices and results. The Warning Letter detailed two main areas of interest:

1. "There was a failure to establish written control procedures to monitor the output and validate the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product . [21 C.F.R. § 211.110(a)]. In addition, there was a failure of the quality control unit to reject in-process materials during the production process that were tested for identity, strength, quality and purity . [21 C.F.R. § 211.110(c)]."
2. "There was a failure to thoroughly investigate any unexplained discrepancy or the failure of a batch or any of its components to meet any of its specifications whether or not the batch has already been distributed [21 C.F.R. § 211.192]."

## Concern #1

The basic issue in concern #1 was that there were apparently repeated examples of lots failing to meet finished product specifications for endotoxin limits (this was an injectable product). Three lots from March 2006 were released to market despite the alleged OOS (Out-of-Specification result). The company did investigate the OOS, and added an in-process endotoxin check to the in-process controls that had an acceptance limit intended to "provide an early indicator of objectionable endotoxin levels and provide pertinent investigational information." FDA alleges that lots exceeding this in-process limit were released to market on the basis of passing finished product testing for endotoxin level. FDA raised two aspects of this practice that were alleged to be violations of cGMP:

> "Your quality control unit should have, during the production process, rejected the in-process materials that had the elevated endotoxin levels."
>
> There was no evidence that endotoxin is uniformly distributed in the vials (the product is an emulsion) and so end-product testing alone is not sufficient, in the opinion of FDA, to demonstrate finished product quality."

Now, apparently the company did not do itself any favors in their response to the situation. Having lots that failed endotoxin testing and apparently establishing an in-process control as a corrective action to that issue, the QC unit seemingly approved a planned removal of the in-process control when batches failed the in-process limits but passed finished product tests. The company argued that the process is robust in terms of endotoxin removal, and the failure of this in-process test does not accurately reflect finished product quality. This might have been more persuasive if the test had not been established as a CAPA for lots that failed finished product testing. In addition, the Warning Letter states that the company felt justified in removing the check as "this in-process testing for en-

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dotoxin was not a commitment in a drug application…" FDA responds: "Please note that in-process controls beyond those described in submissions to FDA are a routine aspect of a firm's quality program to assure compliance with the cGMP regulations. Because we think that it would be appropriate to test your in-process materials for identity, strength, quality, and purity, we believe that eliminating this in-process endotoxin test would be in violation of 21 C.F.R. § 211 .110(c)."

That last sentence is interesting. Is this a general position that FDA is now taking? Are we expected to add in-process checks to ensure conformance to finished product quality *even if they involve established processes and products* or is this in response to a particular case where a company established a control in an attempt of corrective action and then recommended removing it when the control apparently became less useful? It is, of course, not clear from this one instance but this will be interesting to follow.

## Concern #2

The FDA raised concerns over the investigations of the company into failure of a batch or any of its components to meet specifications. The FDA pointed out that multiple lots significantly exceeded in-process levels for endotoxin, but as they later passed finished product release testing the Quality Control Unit permitted their release without determining the source of the contamination. There was an investigation performed, but it concluded "that since the in-process test for endotoxin was for investigational purposes, full investigations were not required."

The company contends that the process is robust in endotoxin removal, and so failure of the in-process check (which the company established to address finished product OOS endotoxin levels in finished product lots) is immaterial. In response to the 483 observations which preceded the Warning Letter the company conducted a couple of studies that FDA acknowledges, but rejects:

"Your studies showed that filtration did not reduce the levels of endotoxin.

Your studies were not sufficiently representative of production conditions and did not provide for a safety margin. For example, they were conducted using a minimum load, rather than a production load. Acceptance criteria should have a safety margin built in to provide adequate process assurance (analogous to depyrogenation validation studies, which demonstrate a minimum three log endotoxin reduction) . A study that demonstrates only a XXX log endotoxin reduction under production conditions would be insufficient to support

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passage removed from the national repository. The broth culture is then prepared for cryopreservation (see "Cryopreservation of Bacteria" by Liz Kerrigan that appeared in the February 2007 issue of *PMF News*). The prepared culture can be (should be) split into many different vials which are frozen individually. Standard techniques for this freezing step can be found from ASTM (1997). Note that the long-term viability of these cultures is not clear for all storage conditions. Therefore, viability checks should be run on standard strains annually.

The next passage for the organism occurs when the frozen vial is removed and streaked on nutrient plates or tube slants. This second passage is acceptable for growth promotion studies, but there is evidence in the literature of the effect of inoculum preparation variables affecting results of some tests (see Boomfield 1995, Gilbert 1987, Orth 1989). If you wish to pass the cultures once (recommended for most tests) or twice more (recommended for the antimicrobial effectiveness test) you still are only 4 passages removed from the national repository strain.

## Generation of a Pure Culture

An assumption of most QC microbiology assays is that we are working with a pure culture of the correct organism. We put media under incoming quarantine and release procedures, why shoulder not do the same for the challenge organisms we use (Sutton, 2007). This is, in fact, recommended by USP (USP, 2008). The best place to withdraw a sample for testing is at the first passage where you have a lab batch ready for cryopreservation. This should be tested by streaking for single colonies (see PMF Newsletter of December, 2006) and checking for purity of the culture, the identity of the microorganism, and confirmation that the new lot is the same strain as the ones previously used (genotypic methods?).

Having established the purity and identity of the batch grown and placed in cryopreservation, this state must be protected. Most of us can pull out the frozen vial, sterilize a loop and take a sample without contaminating that frozen vial. However, it is impossible to prove that we did not do that in a situation where contamination crept into the process at some point. It is therefore recommended to discard each frozen "Cryo" stock vial after sampling. Similarly, once a container along the chain (plate or slant tube) is sampled, that container should be considered contaminated and discarded. The purity of the inoculum should be confirmed from the inoculum preparation as the final check in this process.

## Generation of a Traceable Culture

Ideally we can trace the inoculum used in a test from that test all the way back to receipt of the shipment

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from the national repository and then back forward to all tests that used this particular shipment. This is very possible if all incoming microorganisms are assigned identification numbers for this purpose, and all sub-batches are traced using a reasonable system. The value of this system in eliminating the culture (clear evidence of purity, lack of problems in other tests that used the culture, etc) make the effort involved in tracing the cultures.

One method that is straightforward to execute is to assign the incoming microorganism ID number, then a second 3-digit number is added after a decimal point to identify the "Cryo" vial, then another decimal point followed by a 2-digit number to identify the working stock preparation followed (finally) by a T1 or T2 to denote the first or second transfer from that working stock. This leads to long, but precise, identification numbers. For example, the culture batch number 12345.333.22.T2 would identify the inoculum batch as the second transfer from a microorganism that had been assigned the identification code "12345" upon receipt and then stored in the "cryo" tube "333" before being streaked as the working culture preparation identified as "22". Obviously, for this system to work quality checks (of some sort) and records of each step of the seed lot procedure would be required. Thi s method of tracking the cultures is only one of many that would be suitable for the purpose.

## Conclusions

The use of a well-designed seed lot culture technique can provide many benefits to a regulated lab (or for that matter a non-regulated lab). If implemented and executed with an eye to making it part of the overall Quality system for the lab, this seed lot technique and its associated records can help in assuring pure cultures for inocula that are traceable if need be for investigations. In addition, the system can generate a large supply of fresh cultures, none of which exceed 5 passages from the original shipment from the national repository.

## References

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release of XXXXX lots that have high in-process endotoxin loads."

The letter gets a little alarming from the company's perspective as FDA states "In light of the high endotoxin test results seen at your facility during the manufacturing of XXXXX, we are concerned that your firm may lack an adequate understanding of the product and the process for manufacturing XXXX." Later FDA adds the boilerplate warning "You should take prompt action to correct the violations cited in this letter. Failure to promptly correct these violations may result in legal action without further notice, including, without limitation, seizure and injunction."

## Concluding Comments

There were other issues cited in the Warning Letter, but as they really did not involve microbiological control issues we have not discussed them. I found this Warning Letter interesting for several reasons. First of all, it should be apparent that if you establish an in-process control specification in response to an OOS situation, you really need to pay attention to the consequences of that decision. Having established the control as a Quality (capital "Q") measure, you then must abide by the results of the test or determine an extraordinarily persuasive reason to set the results aside. Also of interest is the assertion by FDA that in-process controls are required to assure finished product quality. This latter point is, of course, not new. However, the degree of emphasis placed on the point by FDA is telling. Finally, if a company wishes to argue that their process is robust in removing a contaminant, adequate and compelling data is required to demonstrate that fact (particularly if the process has yielded contaminated lots in the past).

All in all, a very interesting warning letter that can be downloaded at http://www.fda.gov/foi/warning_letters/s6579c.pdf

# From the News Wire

## FDA Advisors Declare 'FDA Science and Mission at Risk'

Broad-ranging Report Concludes that Increased Resources Are An Essential First Step

WASHINGTON, Dec. 3 /PRNewswire-USNewswire/ -- The nation's food supply is at risk, as are the regulatory systems that oversee the nation's drug and device supplies, according to a subcommittee of the FDA's Science Board in a report to be presented today. The subcommittee attributes the deficiencies to soaring demands on the FDA; and resources that have not increased in proportion to those demands. They conclude that "this imbalance is imposing a significant risk to the integrity of the food, drug, cosmetic and device regulatory system, and hence the safety of the public."

The result of a year-long review by a distinguished panel of experts, the Subcommittee's 300-page report concludes that the state of FDA's scientific and regulatory programs could not be separated from the lack of resources. It urged funds to support the agency's scientific base, hire a broadly-capable scientific workforce, and build a sophisticated, modern information technology infrastructure.

The report has been posted on the FDA website at: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4329b_02_00_index.html.

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## Committee Reviews FDA Report

Without a significant and sustained increase in funding, the FDA cannot fulfill its mission to protect the public health, a subcommittee of the FDA's Science Board says in a report detailing the agency's shortcomings.

A lack of resources, weak scientific base and obsolete IT system are hindering the FDA, the Subcommittee on Science and Technology says in the report discussed at a Science Board meeting Dec. 3. Most of the problems are due to a dearth in funding, according to the report.

The Science Board agreed by voice vote to accept the subcommittee's report and take steps to provide further review of high priority scientific programs and the scientific capacity and processes of the agency's Office of Regulatory Affairs.

The subcommittee was cautioned against assessing the FDA's financial resources but could not review the agency's shortcomings without discussing resources, subcommittee Chairwoman Gail Cassell said.

FDA Commissioner Andrew von Eschenbach said the agency is undergoing enormous change and must continue to adapt to be successful. Two years ago, the agency was "stressed and stretched" but is now making progress, he added.

Much of the change comes from the FDA Amendments Act (FDAAA), which included more than 200 initiatives the agency can carry out, including the ability to require safety-related label changes and postmarketing studies or clinical trials.

The new authorities are not yet in place, and the FDA must determine which programs will require additional guidances or development of new regulations, FDA Deputy Commissioner Janet Woodcock said.

The FDA needs more employees to focus on postmarketing issues and also will need many new policies and procedures, particularly in clarifying the relationship between the Office of New Drugs and the Office of Surveillance and Epidemiology, Woodcock added.

The subcommittee's report can be seen at www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4329b_02_01_FDA%20Report%20on%20Science%20and%20Technology.pdf
www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4329b_02_01_FDA%20Report%20on%20Science%20and%20Technology.pdf

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## Upcoming Events

**February 18-19 2008** Open Conference on Compendial Harmonization.
Baltimore, MD

**March** - Environmental Monitoring (Susan Schniepp, moderating)

**April** - Validation Issues in Microbiology
Philadelphia, PA

**May** - Microbiology Investigations (Frank Settineri, moderating)

**June** - GMP for the Microbiology Lab (Scott Sutton, moderating)
Dallas/Ft. Worth, TX

**September** - Cosmetic Microbiology
Newark, NJ

**October** - 2008 PMF Fall Forum (Scott Sutton, moderating)
Rochester, NY

**November** - Bacterial Endotoxin Summit (Karen McCullough, moderating)
New Brunswick, NJ

**Offering In-House Courses on Microbiology/Aseptic Processing:**

- The Microbiology Network/High Peaks Associates http://www.highpeaks.us/in-house.htm
  Experienced in custom-designed courses for the lab or the manufacturing facility, including
  - GMP for the Microbiology Lab
  - Microbiology for Manufacturing
  - Microbiology for Management
  - Auditing the Microbiology Function
  - Investigating Microbiological Data Deviations
- 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.



### Discussion List Update

**PMFList:**
Number of Subscribers: 2736
Number of Countries: 63
Number of Messages Last Month: 232

**PSDGList** (Pharma Stability Discussion Group):
Number of Subscribers: 1064
Number of Countries: 23

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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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## The National Collection of Pathogenic Fungi

The National Collection of Pathogenic Fungi (NCPF) is the United Kingdom's only culture collection specialising in fungi pathogenic to humans and animals. Since its inception at the London School of Hygiene and Tropical Medicine in 1946 the NCPF has been an integral part of the Mycology Reference Laboratory which means that the expertise is available on site for strain authentication. At present the collection holds about 2500 strains, most of which are available for sale and distribution.

Current holdings include:

- Approximately 500 strains of dermatophytes and related organisms from the UK and abroad from human and animal infections including many original type strains
- Approximately 900 mould strains from sub-cutaneous and deep-seated human and animal infection
- Approximately 900 pathogenic yeast strains
- Approximately 140 strains of dimorphic fungal pathogens

The NCPF maintains close links with its sister collection NCTC at HPA Colindale where all the lyophilisation of NCPF strains is conducted.

More information regarding the NCPF can be found at http://www.pharmaceutical-technology.com/contractors/consult/ecacc/

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## The National Collection of Type Cultures: ECACC's New Website

September 2007 saw the launch of a new NCTC website, which provides easy access to over 5000 reference bacterial cultures, 100 mycoplasmas and more than 500 plasmids, host strains, bacteriophages and transposons. All of these are of medical, scientific and veterinary importance. In addition, the website offers valuable comprehensive resources to customers wishing to search for specific bacterial cultures, their characteristics and DNA sequences together with frequently asked questions, technical support and significant bibliographies. Polyphasic identifications can be performed and nomenclature reviewed.

The website will be regularly updated with new bacterial strains and news from the NCTC. This will allow customers to easily access specific information or alternatively gain a general understanding of what NCTC can offer," explains Dr Elizabeth Fashola-Stone, project manager, marketing.

NCTC was established in 1920 and is the oldest collection of bacterial cultures in the world to offer a supply service. This continues as its prime function today, supplying reference bacterial cultures worldwide to support academic, health, food, veterinary institutes and commercial organisations.

In addition to supplying bacterial cultures, NCTC also provides a range of associated services, which includes:

- Freeze-drying of customer-provided strains
- International Depository Authority (IDA) patent depository for bacterial cultures
- Production of cultures and samples for use in European Quality Assurance (EQA) schemes
- Provision of cultures (in LENTICULE disc format) with defined numbers of cfu/disc

The NCTC is one of the Health Protection Agency Culture Collections together with:

- European Collection of Cell Cultures (ECACC)
- National Collection of Pathogenic Fungi (NCPF)
- National Collection of Pathogenic Viruses (NCPV)

The Health Protection Agency Culture Collections are a strategic business unit within the Health Protection Agency.

**For more information:**
http://www.pharmaceutical-technology.com/contractors/consult/ecacc/