Microbiological Best Laboratory Practices, USP <1117> Value and Recent Changes to a Guidance of Quality Laboratory Practices

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The field of pharmaceutical microbiology is responsible for many key objectives in ensuring patient safety and product quality. Quality control, method development, process and product design, and product stability are a few of the objectives. The United States Pharmacopeia (USP), other global pharmacopeias and some parallel industry specific compendia offer some standardized test methodologies and material specifications relating to microbiological quality and control. However these test methods assume significant operational knowledge on the part of the laboratory practitioner and significant operational capabilities of the laboratory itself. It is imperative to have some basic knowledge, experience and infrastructure that can support consistent use of these methods. The USP informational chapter <1117> Microbiology Best Laboratory Practices was developed to serve a part of this purpose.

The proposed general information chapter about Microbiological Best Lab Practices was first published in 2003 (USP 2003) in the *Pharmacopeial Forum*, following the long standing USP Revision process of development and writing standards by experts along with public comment. After comments and further revision of the draft chapter (USP 2004), it was first published as an official USP Informational chapter in USP 29, 3 years later (USP 2006).

The intent of the chapter was to address a perceived lack of clarity on the parts of both industry and the regulators on the basic requirements of infrastructure needed to support mandatory microbiological criteria and tests in the USP. Chemistry had a lot of guidance and information, but there was very little guidance for microbiological testing.

The question of laboratory variability was central to this concern. Microbiologists work every day with variability in the detection, recovery and growth of microbiological species. This variability can be thought of in two categories, "avoidable" variability (variability due to poor practice) and inherently unavoidable variability (variability due to limitations of the methods and the vagaries of dealing with biological samples - see Jarvis, 1989). The goal of "best practices" would then, be to minimize "avoidable" microbiological error.

What are 'Best Laboratory Practices' in Microbiology?

Well, they are a way of developing control or, in analytical terms, having a 'system suitability' of the laboratory. It makes sense. Using and benchmarking best laboratory practices plus good documentation practices ensures reliability of data. In our highly regulated industry, assurance is paramount to control.

Note: we are not saying anything about Good Manufacturing Practices (GMPs) – although they certainly have a place in the discussion! We are talking about laboratory quality and best practice that goes well beyond 21 CFR (Code of Federal Regulations) whatever (211, 612, 820, etc). The goal of this is to minimize variability and erroneous results. A secondary benefit is to provide a benchmark for the laboratory and for auditors of laboratory functions. Also, variability, inherent in microbiology, means that microbiological data deviations will happen.

What's in the Original Chapter?

Let's take a look at the original USP chapter and its topics of discussion. Figure 1 shows the integration of all the key topics:

- Media preparation
- Microbiological cultures
- Lab equipment
- Laboratory layout
- Lab records
- Interpretation of results
- Training
- Documentation

In the section about media preparation, the discussion included media preparation, media storage, and quality control testing of media.

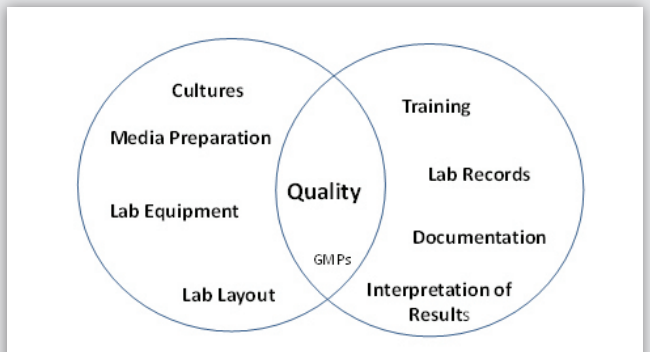

Figure 1.

The Original Chapter

Media Preparation and Quality Control

The quality of work in a microbiological laboratory depends on the quality of the culture media. It is essential to use the correct media for the purpose at hand, although the correct media is not always obvious. For example, water testing is commonly performed with R2A agar, but many facilities use TSA (Trypticase Soy Agar) or HPCA (Heterotrophic Plate Count Agar) for this purpose. The recommendation is provided that the choice of media should be consistent, appropriate and justified.

An entire section is devoted to the question of media storage and the effects this might have on the media quality. Excesses of heat and cold are to be guarded against, as is the potential for dehydration of poured plates. Some guidance is also provided in quality control for molten media used in pour plates.

Maintenance of Microbial Cultures

Second only to media, safeguarding the stock cultures is the most important component of a successful microbiology laboratory. These must be handled carefully at all times to avoid contamination.

The care of the cultures starts upon receipt. A careful stock culture curator will confirm the identity of the received cultures, even if they come from as respected a source as a national culture collection. Mistakes can happen. The use of an incorrect strain in a compendial test could bring the results of weeks or months of work into question.

The chapter reinforces the compendial recommendation for the "seed lot technique" in culture maintenance. Critical to this is the need to go into your containers of stock culture only once, and in restricting the number of passages. Now, it must be stated that there is nothing magic about the number 5. This number of passages gained popularity in the compendia through its use in the Sterility Test, and has been maintained for consistency. The point to the practice is that a careful lab will safeguard the purity and identity of their stock cultures by limiting the potential for "drift" due to excessive transfers.

Maintenance of Laboratory Equipment

This section was originally included more for the sake of completeness than because of concerns peculiar to the microbiology laboratory. Basic information on qualification requirements, documentation, etc. was included.

Laboratory Layout and Operations

The need for this section stems from the concern that too few facilities understand or plan for the separation of samples from a microbiological perspective. The success of a laboratory can be enhanced by the thoughtful separation of samples likely to have contamination from those that are expected to be sterile.

Training of Personnel

The chapter states plainly what should be common sense in recommending that microbiologists and managers in the pharmaceutical support lab should have academic training in microbiology or allied health sciences. This recommendation is in line with current best practice for biosafety as laid out in the 5th Edition of the Center for Disease Control's (CDC) manual "Biosafety in Microbiological and Biomedical Laboratories (BMBL)." (CDC, 2007)

Documentation and Maintenance of Laboratory Records

These sections were included only for the sake of completeness, although additional "GMP Rules" are added in this revision. It is nice to see the rules written down somewhere.

One aspect of these sections should also be addressed, and that is the expectations when dealing with contract laboratories. The list of required bits of information for the lab write-up is designed to provide a minimal amount of proactive documentation for GMP requirements. This is also a reasonable expectation for "GMP" studies from contract laboratories. Many labs will accept little more than summary reports from the contract lab; it is the opinion of the authors that this is an ill-reasoned position as it prevents adequate QAU (Quality Assurance Unit) review of the study as required by 21 CFR210.3(b)12 & 15, 21 CFR211.84, 21 CFR211.87, 21 CFR 211.160 and 21 CFR211.165. While it might be argued that the contract lab's QAU fulfills this requirement, this assumes that the client has complete and total confidence that the current Quality procedures and policies of the contract lab meet or exceed their own.

Interpretation of Assay Results

This section was initially envisioned to provide information on laboratory investigations. However, during the writing process it became clear that the scope of this section was broader than merely investigations, and so the current title was settled upon as the best choice.

A discussion of the inherent variability of microbiological data was necessary in this chapter. One view of good laboratory practices could be structured around determining practices that minimize variability in the microbiology lab. However, because we are dealing with such low numbers on plates (frequently less than 20 CFU/plate) and the real opportunities for human error in tests that may run over a month to completion, the microbiologist must always be aware of the role that random chance has in the data and be on guard against over-interpreting the results of a study.

Revisions in the Current Version

Chapter <1117> is a living informational reference, which means that as the expert committee sees or hears of potential improvements, the chapter can be updated. Since the official chapter was first published, and as part of a quality improvement plan for a USP chapter, both expert committee and comments from the public already have led to some changes, in order to:

- Keep the chapter updated
- Improve clarity
- Add more information that the public requested

Now, let's take a look at the newly revised chapter, in Figure 2.

Some new relevant topics were added: Lab Resources, Sample Handling, and Media Incubation Times.

In addition to the new topics, some modifications were made to the language used to improve clarity and accuracy within these topics.

More Detail of the Recent Changes

Introduction

Some clarifications were added to the Introduction. The sentence about key parameters relating to equipment was modified, using the word 'operation' along with 'control' to indicate the importance of equipment performance. When discussing data variability, as mentioned earlier, we replaced the word 'known' with 'inherent' to enhance clarity about the risk of variability in microbiology.

Media Preparation and Quality Control Testing

The revised chapter expands the discussion of media preparation as well. The recommendations include accurate weighing of dehydrated components, the use of high-quality (USP Purified) water, as the first intent choice, completely dissolving the dehydrated media or individual ingredients, and the need to control the heating of the media to avoid damaging heat-labile components of the media. Some recommendations on the labeling and packaging of media are also provided. A general change in the chapter is apparent in this section, with cross-references to various other relevant USP chapters added. This cross-referencing also raises expectations that the microbiology lab will be familiar and compliant with these other chapters as well. For instance, instead of explaining how to calibrate a balance, a reference was added for the USP General chapter about *Weighing on an Analytical Balance*, USP <1251>.

The quality control of the media is a critical concern. Interestingly, initially some of the most passionate commentary on the chapter dealt with the "excessive" amount of space provided to media quality checks. Since the initial release in 2003, however, the harmonized Sterility Tests and the harmonized Microbial Limits Tests have both incorporated stringent media quality checks. This section provides an opportunity to provide additional general information on media growth promotion testing which led to a significant expansion in revision.

The two statements about Sterility Assurance Level (SAL) related to media sterilization were removed. The removal of SAL in both cases relates to a belief that SAL was not developed for use with media sterilization.

Clarifying the intent of the discussion about media sterilization, the 'container size' parameter was added as a key part describing what can affect the rate of heating during a sterilization cycle. As a consequence

of sterilizing or heating conditions that can have multiple effects on media, the discussion was enhanced by adding to the physical effects parameters another issue, the potential of reduced growth promotion or selective activity of a medium.

The chapter now clarifies what is meant by room temperature when testing pH, by the addition of 20°-25°C. In addition, a reference was added for USP <791> which discusses pH measurement and calibration. As we know, there is an increased use of purchased media in our new world of Lean Labs! A statement about purchased media was added relating to the common storage at refrigerated temperatures before pH testing.

The recommendation of Quality Control (QC) testing was updated to include **all** prepared media. Because of the new harmonized pharmacopeial chapters for *Microbiological Enumeration* <61>and *Specified Microorganisms* <62>, information was added to state the key indicated parameters for QC testing of media:

- pH
- growth promotion
- inhibition and indicative properties

This was meant to align better with these harmonized chapters.

The selection of challenge microorganisms for QC testing of media is stated in the chapter. To clarify how selection is determined, a statement was rewritten to indicate relevance, use and selection of the growth promotion microorganisms, in particular environmental isolates.

Another sentence was added to the discussion to help clear up the response relating to Growth Promotion failures. Also added was a statement that any failed Growth Promotion tests would not negate any positive recovery that occurs during testing.

Media used in aseptic or clean areas were recommended to be 100% pre-incubated and inspected first, then followed by growth promotion testing.

Microbiological Cultures

Since the viability and identification of cultures used for controls and QC testing are critical, this section intends to recommend parameters to ensure integrity is the objective. We all often use cultures that are redistributed or reworked from primary collections by secondary suppliers. So, it is important to qualify the secondary suppliers. This statement was made twice in this section to stress its importance.

The discussion on microbial cultures was expanded in this revision to make allowance for "ready-to-use" cultures and qualified secondary suppliers. A discussion of the reasons for minimizing the microbial passages involved in culture maintenance was included in response to some concerns from the field.

In this modern day of using polyphasic approaches to identification, the original statement of 'accepting only genotyping' was changed to restate the main intent –To determine purity and identity by your own choice of approach.

A clarification was made to define the type of change that can occur due to increased transferring of cultures.

Lab Equipment

Originally, this section had a title of Maintenance of Lab Equipment. The new section title, Lab Equipment, allows for more general recommendations instead of just maintenance (such as cleaning, calibration, and use). Thus, there is some new discussion about equipment cleaning and sanitization and autoclave validation related only to media.

Sanitization of equipment includes a statement about regular cleaning of key routine equipment where growth conditions can occur, and that equipment which is difficult to sanitize should be dedicated for specific use situations.

Most lab equipment in the microbiology laboratory is subject to the standard validation practices of IQ, OQ, and PQ (Installation Qualification, Operational Qualification, and Process Qualification). As is common, periodic calibration/maintenance may be required for the particular equipment based on its nature, and performance verification checks should also be performed regularly. The frequency will depend on characteristics and use of the equipment (further information can be found in USP <1058> Analytical Instrument Qualification).

Laboratory Layout

Laboratory layout was an interesting part of this chapter. It was originally written to talk about separation of clean and 'dirty' activities, in microbiological terms, as well as containment and sufficiency of space for activities. From this section, a new topic was extracted, that of Sample Handling.

Sample Handling

This new section discusses sample sensitivity, storage and transport. The importance of using aseptic techniques for all sampling activities is a necessary addition in this section. The appropriate marking of samples to enhance tracking from source to lab is also included here.

This short section was added in response to questions about the relevance of samples that are stored for extended periods before testing, or are transported to a distant facility for testing. As the test system is a living system, it is expected to react to stimuli over time. The effects of storage over time could have a significant effect on the test results.

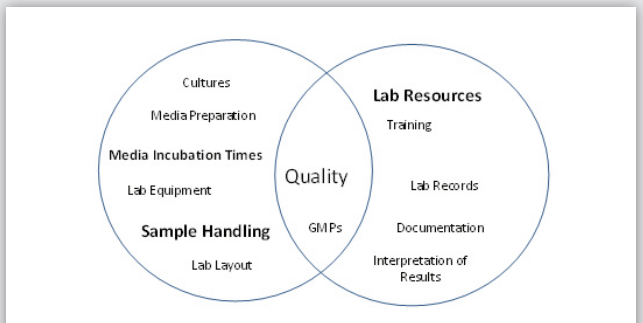

Figure 2.

Microbiological Media Incubation Times

A new section was requested in the public comments to offer an approach to answer the question, "How do we determine end points of incubation schemes?"

Despite the section's small size, it has the potential for a large impact on the processes in the lab as it provides recommendations for determining how to interpret incubation times. These recommendations would encourage documentation of "time-in" and "time-out" proactive documentation of the incubator usage. This short section is reproduced below:

"Incubation times for microbiological tests of less than 3 days' duration should be expressed in hours: e.g., "Incubate at 30° to 35° for 18 to 72 hours." Tests longer than 72 hours' duration should be expressed in days: e.g., "Incubate at 30° to 35° for 3 to 5 days." For incubation times expressed in hours, incubate for the minimum specified time, and exercise good microbiological judgment when exceeding the incubation time. For incubation times expressed in days, incubations started in the morning or afternoon should generally be concluded at that same time of day."

The purpose of these rules to consider is to simplify and clarify a response to this ongoing question.

Training of Personnel

Personnel training is a core parameter of our laboratories. Training is one of the means of developing competency, and it may include achieving certification by an accredited body. The importance of this parameter is underscored by 21 CFR 211.25 "Personnel Qualifications."

Since 'competency' is becoming a buzzword in our industry, and microbiologists do have options to prove their competency, this discussion is important to develop ways of meeting the regulatory inspection requests as well as the quality improvement approaches of the laboratories. One option for proof of competency is the NRCM (National Registry of Certified Microbiologists, American Society for Microbiology) certification. The aspect of microbiology education and training, theoretical and practical, being unique is strongly stated in comparison to chemistry training.

This position is expanded with specific instruction on the qualifications and competency of the laboratory management. As microbiology is largely operator dependent, it falls on the management to train the operators, review the data and establish procedures. This cannot be done without adequate preparation. Discussing the lab management, a new paragraph specifically addresses this point:

"Competency may be demonstrated by specific course work, relevant experience, and routinely engaging in relevant continuing education. Achieving certification through an accredited body is also a desirable credential. Further, it is expected that laboratory supervisors and managers have a demonstrated level of competence in microbiology at least as high as those they supervise. Expertise in microbiology can be achieved by a variety of routes in addition to academic course work and accreditation. Each company is expected to evaluate the credentials of those responsible for designing, implementing, and operating the microbiology program. Companies can thus ensure that those responsible for the program understand the basic principles of microbiology, can interpret guidelines and regulations based on good science, and have access to individuals with theoretical and practical knowledge in microbiology to provide assistance in areas in which the persons responsible for the program may not have adequate knowledge and understanding. It should be noted that microbiology is a scientifically based discipline that deals with biological principles substantially different from those of analytical chemistry and engineering disciplines. Many times it is difficult for individuals without specific microbiological training to make the transition."

In addition to the recommendation that the microbiology staff have studied a relevant subject while in school, the proposed guidance chapter points out a fundamental link between training and the unit's SOP system. It recommends that the SOP system should be comprehensive and serve as basis of the training program. This proposal also recommends that performance assessments be done periodically and should demonstrate competency in core activities of the lab.

Another area that impacts indirectly, but often, on laboratory competency and capability is resourcing. This new section was added to mention the issues revolving around Resourcing.

Laboratory Resources

The importance of adequate resourcing cannot be overstated to best lab practices. This is reinforced in 21 CFR 211.22(b), 211.25(c), it was felt that including skills in budgeting and investigations were important for Supervisors of Microbiology labs. Thus, in this new section, the importance of budget management and investigational skills is discussed.

Interpretation of Assay Results

The additions in this section are thoughts that are not found in any of the 'mandatory' general Microbiology chapters. Expansions on lab investigations are provided, as is a formal recognition of the phrase "Microbiological Data Deviation" (MDD).

This section of the proposed guidance document is intended to be both a discussion of the limitations of compendial test methodologies and a guide to developing methods of investigating test failures. It discusses the difference between a test that has failed, a test that should be invalidated and a test that should be repeated for confirmation.

Summary

All the changes mentioned above are beneficial to practice and understanding microbiology in its role in quality assessment.

Restating the intent of the chapter, it is: To develop and ensure microbiological laboratory effectiveness and data integrity.

The original version published in 2006 was a good start on a difficult subject, the revision published in 2010 expands on this work in several critical areas. The laboratory should pay particular attention to the information in this USP chapter not only for their internal policies, but also as an aid to the audit and review of contract laboratories.

This general information chapter has been used successfully to improve the effectiveness, efficiency and inspection-readiness of a Microbiology Laboratory. In these days of lean organizations, high performance and consistency of a laboratory can lead to sustainability. The chapter is yours to gain the most benefit as a benchmark.

Acknowledgements

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Author Biographies

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Scott Sutton has over 25 years of experience in the the pharmaceutical, medical device, cosmetics and personal products industries with extensive publications and presentations. Consulting and training in GMP, contamination control, investigations of MDD (OOS), laboratory management and microbiology-related project management are areas of special interest. His clients have included startups, generics, established Fortune 500 companies, law firms and investment broker houses. Scott has owned and operated The Microbiology Network (http://www.microbiol.org) since 1996. This company provides consulting and training services to industry.

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