Regulatory Perspective on Key USP General Chapters in Microbiology

Dennis E. Guilfoyle, Ph.D.

Pharmaceutical Microbiologist

FDA Northeast Regional Laboratory

Disclaimer

The interpretations and emphasis placed on subjects within this presentation are the author's professional opinion and not official FDA and USP positions

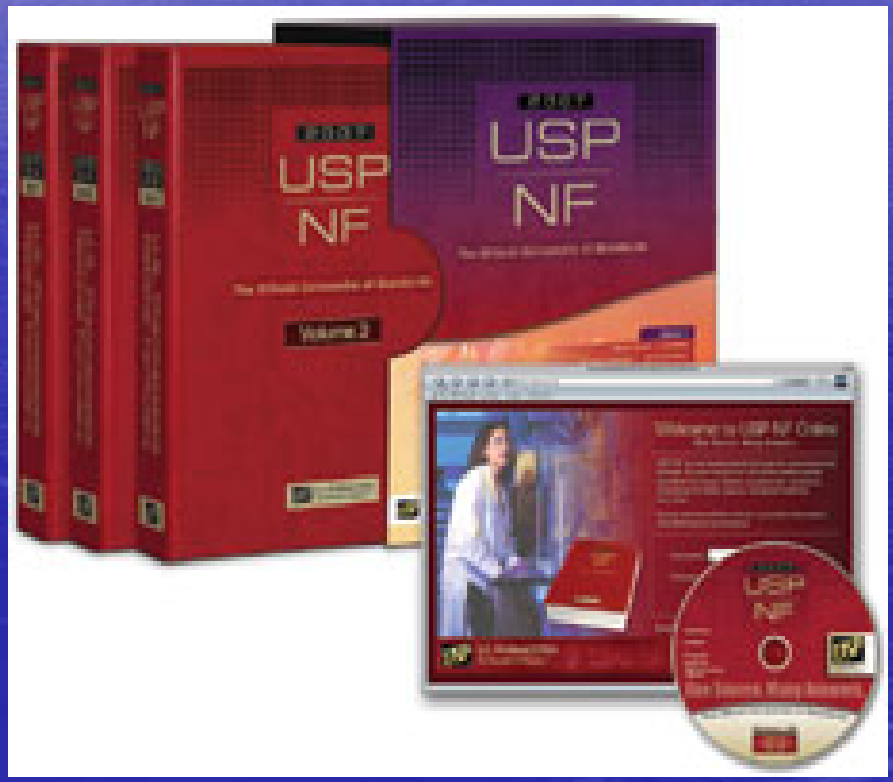
Presentation Outline

1. Introduction; USP and Committee
2. <1111> Microbiological examination of nonsterile products
3. <1112> Application of A_w determination to nonsterile pharmaceutical products
4. <1211> Sterilization and sterility assurance of compendial articles
5. <1113> Microbial characterization, Id, and Strain Typing
6. <1117> Microbiological best laboratory (optional)

What is the US Pharmacopeia?

- Independent organization
- Book of definitions and requirements

- The first edition was published on Dec 15, 1820. It was essentially a “recipe book” for 217 drugs.



There may have been a non genetic excuse for Great Great Grandpa acting *differently*

More Medicine

Who am I?

The USP establishes

- Titles
- Definitions
- Descriptions
- Standards for Identity, Quality, Strength, Purity, Packaging and Labeling
- It is an “evolving” document

USP : Legal Status of the Official Compendia

- Three references to the USP are found in several sections of the Federal FD&C Act.
- FDA may enforce compliance with official standards in USP-NF under the adulteration and misbranding provisions of the FD&C Act.

General Chapters

- REGULATORY: First section- “General Tests and Assays” Chapters are numbered from <1> to <999>
- INFORMATIONAL (NOT a requirement): Second section “General Information” Chapters are numbered from <1000> to <1999> Expert advice; general and detailed chapters (13-primarily Micro)

USP Microbiology and Sterility Assurance Expert Committee 2010-2015 (new cycle)

- James Akers, Chairman
- Scott Sutton
- James Agalloco
- Dilip Ashtekar
- Anthony Cundell
- Karen McCullough
- Dennis Guilfoyle,FDA liaison
- David Hussong, FDA liaison
- Russell Madsen
- Jianghong Meng
- Leonard Mestrandrea
- Rainer Newman
- Donald Singer
- Edward Tisdwell
- Mickey Parish, FDA liaison
- Radha Tirumalai USP Staff liaison

Areas of Responsibility

- Microbiological test methods and assays
- Microbiological Monitoring
- Not Responsible for:
  - *Water*
  - Antibiotics
  - Monographs (other than setting Microbial Limits, Sterility and Bacterial endotoxin specifications)

Let's Begin

New USP Microbiology Informational Chapters- Since 2005 –

<1072> *Disinfectants and Antiseptics*

<1112> *Application of Water Activity Determinations to Non-sterile Pharmaceutical Products*

<1117> *Microbiological Best Laboratory Practices*

<1223> *Validation of Alternative Microbiological Methods*

Revised USP Microbiology Informational Chapters Since 2005-

<1072> *Disinfectants and Antiseptics*

<1111> *Microbiological examination of nonsterile products: Acceptance criteria for Pharmaceutical preparations and substances for Pharmaceutical use*

<1117> *Microbiological Best Laboratory Practices*

<1208> *Sterility Testing-Validation of Isolator Systems*

<1211> *Sterilization & Sterility Assurance of Compendial Articles*

<1222> *Terminally Sterilized Pharmaceutical Products-Parametric Release*

Proposed Revisions and New Chapters

<1113> Microbial Identification

Revised New Chapter Proposal to be published in PF 36(6) Nov-Dec 2010 Issue

<1116> Microbiological Evaluation of Clean Rooms and Other Control Environments

Revision to be published in PF 36(6) Nov-Dec 2010 Issue

In the Pipeline of Microbiology EC……..

New Informational Chapter Proposals

<XXXX> Contamination Control in Non-Sterile Product Manufacturing

<XXXX> Isolators for Aseptic Processing

<1211> Sterilization & Sterility Assurance of Compendial Articles

Long Term fix

- Future chapters will address sterilization at an introduction level only section (discussed in later slides)

So how do I really feel...

<1111>

Microbiological Examination of Nonsterile products:

Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical use

<1111>: Acceptance criteria for Pharmaceutical preparations...

- Table 1 Acceptance Criteria for Microbiological Quality of Nonsterile Dosage forms based on route of Administration: TAMC, TCYM, specified microorganisms
  - Oral- aqueous and non aqueous
  - Rectal
  - Oromucosal, Cutaneous, Nasal, etc.
  - Vaginal
  - Transdermal
  - Inhalation

  Note: This table is informational not mandatory

<1111>: Acceptance criteria for Pharmaceutical preparations...

"In addition to the microorganisms listed in Table 1, the significance of other microorganisms recovered should be evaluated in terms of the following:

- The use of the product (eye, nose, etc)
- The nature of the product: does it support growth?
- The method of application
- The intended recipient: risk may differ for neonates, infants, the debilitated
- The presence of disease, wounds, organ damage

Where warranted, a risk-based assessment of the relevant factors is conducted by qualified personnel

<1112> Application of Water Activity (A_w) Determination to Nonsterile Pharmaceutical Products- Introduction

<1112> Application of A_w Determination to Nonsterile Pharmaceutical Products- Introduction

"The determination of the water activity of non-sterile pharmaceutical dosage forms aids in the decisions relating to the following:

A. optimizing product formulations to improve antimicrobial effectiveness of preservative systems,

B. reducing the degradation of active pharmaceutical ingredients within product formulations susceptible to chemical hydrolysis,

C. reducing the susceptibility of formulations (especially liquids, ointments, lotions, and creams) to microbial contamination, and...

<1112> Application of A_w Determination to Nonsterile Pharmaceutical Products- Introduction (continued)

D. "Providing a tool for the rationale for reducing the frequency of microbial limit testing and screening for objectionable microorganisms for product release and stability testing using methods contained in the general test chapter *Microbial Enumeration Tests* 61 and *Tests for Specified Microorganisms* 62."

<1112> Application of A_w Determination to Nonsterile Pharmaceutical Products- Introduction

"Reduced microbial limits testing may be justified through risk assessment. This reduction in testing, when justified, may entail forgoing full microbial limits testing, implementing skip-lot testing, or eliminating routine testing."

- Be ***very*** cautious with this suggestion

<1112> Application of A_w Determination to Nonsterile Pharmaceutical Products- Introduction

"When formulating an aqueous oral or topical dosage form, candidate formulations should be evaluated for water activity so that the drug product may be self-preserving, if possible. For example, small changes in the concentration of sodium chloride, sucrose, alcohol, propylene glycol, or glycerin in a formulation may result in the creation of a drug product with a lower water activity that can discourage the proliferation of microorganisms in the product."

<1211> Sterilization and Sterility Assurance of Compendial Articles

<1211> Other Completed Activities

- Eliminated the entire discussion of sterility testing at the conclusion of the chapter. The only content in USP relative to sterility tests will be in the harmonized <71>.
- Eliminated the older radiation sterilization guidance & directed readers to ISO standards.
- Some comments were received indicating we hadn't gone far enough.
- That was deliberate on our part. The type of revisions we are contemplating are far more extensive.
- Each method of sterilization will have its own chapter

<1211> The Planned Revision

- Individual chapters on each sterilization method:
  - Aseptic processing as a separate chapter: not strictly a sterilization subject, needs better connection to other chapters
  - New definitions for sterilization validation models. Clarify the role of the biological indicator (BI). Clarify SAL and risk to patient.
  - Integrate Endotoxin Indicator as well as BI & Chemical Indicator content.
  - Proposed new chapters on next slide

Proposed Individual Chapters and New Topics.....

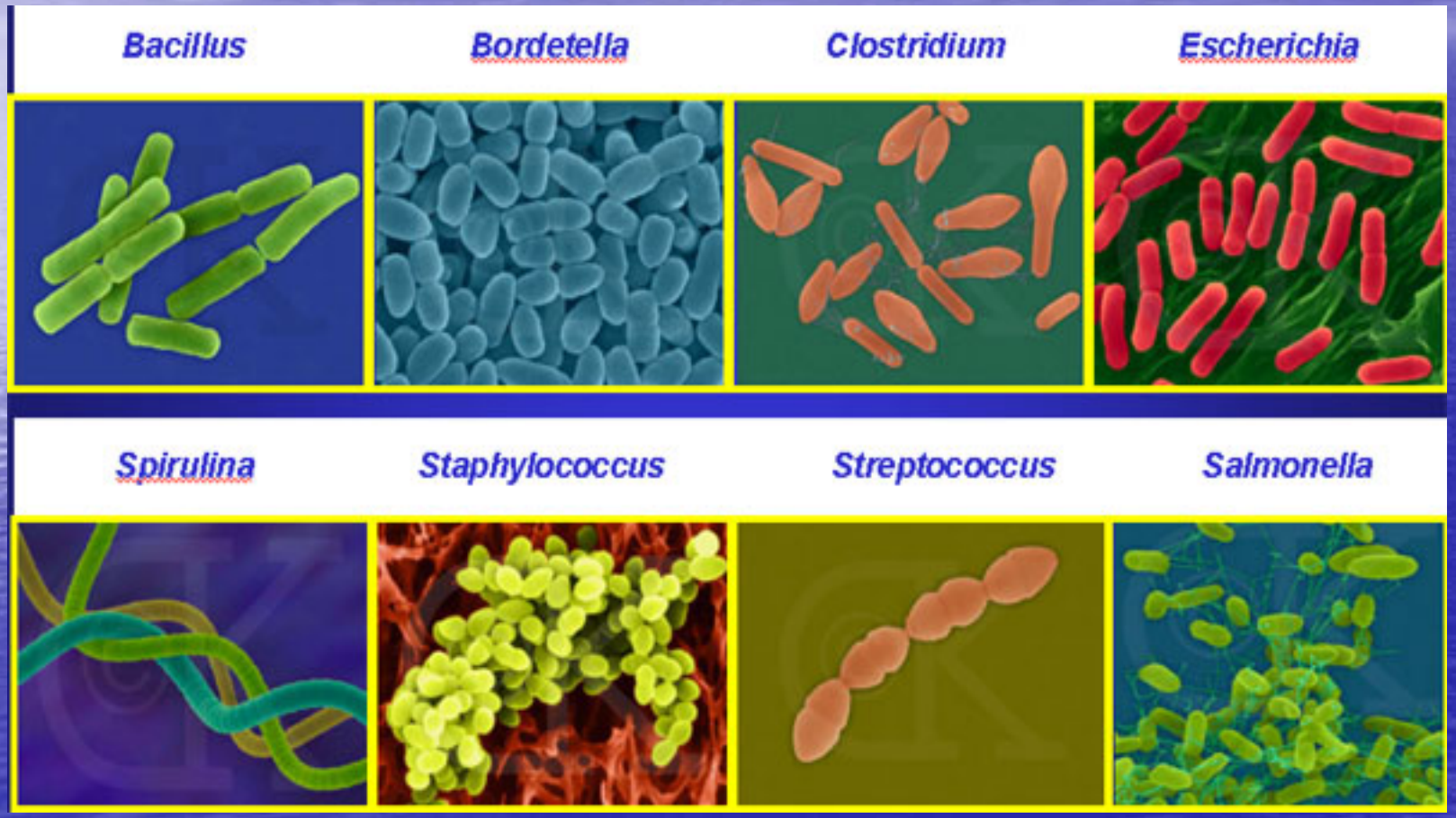
- Chemical Sterilization 

- Dry Heat Depyrogenation
- Dry Heat Sterilization in Ovens 

- Sterilization by Filtration
- Gas Sterilization
- Vapor Sterilization 

- Radiation Sterilization
- Steam Sterilization in Autoclaves
- Terminal Sterilization using Moist Heat

<1113> Microbial Characterization, Identification, and Strain Typing



FDA Guidance: Sterile Drug Products Produced by Aseptic Processing-cGMP, 2004

"Laboratory Controls

- B. Microbiological media and Identification

Characterization of recovered microorganisms provides vital information for the EM program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigator. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine Id of microorganisms to the species (or, where appropriate, genus) level."

FDA Aseptic Guidance, 2004

Laboratory Controls (continue)

- B. Microbiological media and Identification

"At minimum, the program should require species (or, where approp., genus) Id of Microorganisms in ancillary environments (i.e. Class 100, 000) at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitization procedures continue to be effective)"

FDA Aseptic Guidance, 2004

Laboratory Controls (Continue)

- B. Microbiological media and Identification

"Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic technique. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates."

FDA Aseptic Guidance, 2004

XI. Sterility Testing

C. Investigation of Sterility Positives

"An initial positive sterility test would be invalid only in an instance in which microbial growth can be unequivocally ascribed to laboratory error. Only if conclusive and documented evidence clearly shows that the contamination occurred as part of testing should a new test be performed....The investigation's persuasive evidence of the origin of the contamination should be based on lat least the following:"

FDA Aseptic Guidance, 2004

"1. Identification (speciation) of the organism in the sterility test"

"Sterility test isolates should be identified to the species level. Microbiological monitoring data should be reviewed to determine if the organism is also found in laboratory and production environments, personnel, or product bioburden. Advanced identification methods (e.g., nucleic acid based) are valuable for investigational purposes. When comparing results from environmental monitoring and sterility positives, both identifications should be performed using the same methodology"

<71> Sterility testing,
Observation and Interpretation of Results

"The test may be considered invalid only if one or more of the following conditions are fulfilled:

d. After determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure"

<1113> Microbial Characterization, Identification, and Strain Typing

- Introduction
- Isolation of pure cultures
- Preliminary screening of microbial isolates
- Gram staining, spore staining Biochemical screening
- Microbial Identification by Phenotypic methods
- Genotypic Methods
- Verification of Microbial Identification methods
- Phylogenetic Identification
- Glossary

Gram Staining

- Common pitfalls in this method are that heat fixation may cause Gram-positive cells to stain Gram-negative, and older cultures may give Gram-variable reaction. Using too much decolorizer may result in a false Gram-negative result, and not using enough decolorizer may yield a false Gram-positive result.

- One variation that has advantages in some situations is to perform a methanol rather than heat fixation of the bacterial smear. In some cases alcohol fixation may give more consistent Gram stain results. In either method a Gram-positive and a Gram-negative control should be included to allow identification of errors in staining.

Microbial Identification by Phenotypic Methods

- These systems rely on specified culture media and incubation conditions to achieve consistent identification
- freshly isolated stressed microorganisms by subculture from primary recovery may not result in a full expression of phenotypic properties
- Phenotypic microbial identification methods provide information that enables microbiologists to make informed decisions regarding product risk and to recognize changes in environmental microflora.

Genotypic Methods

"Genotypic microbial identification methods are theoretically more reliable because nucleic acid sequences are highly conserved in most microbial species."

"Applicable genotypic methods include DNA–DNA hybridization, PCR, 16S and 23S rRNA sequencing, multilocus sequence typing (MLST), pyrosequencing, DNA probes, and analytical ribotyping."

Genotypic Methods

- Southern hybridization of restriction endonuclease digests is powerful and can be effective in demonstrating differences between two strains. If the banding patterns appear identical this shows only that that restriction endonuclease has similar cleavage sites in that region of the two organisms.
- Demonstration that the two organisms are the same should include two or more different restriction endonuclease digests, each of which yields bands in the area of interest. All bands from the two organisms must be identical.

Verification of Microbial Identification Methods (It's a start)

Microbial identification tests include serological tests, chemical reagents, reference organisms, and instrumentation. The verification of an identification test system can include one of the following:

1) Using an existing system for parallel testing of microbial isolates obtained from routine testing (the number of isolates tested may be as high as 50, and any discrepancies in identification can be arbitrated using a referee method)

Verification of Microbial Identification Methods

2) Testing 12–15 known representative stock cultures of different commonly isolated species for a total of 50 tests

3) Confirming that 20–50 organism identifications, including 15–20 different species, agree with the results of a reference laboratory testing of split sample. In each case the appropriate quality control organisms, as recommended by the supplier and the compendia, should be included in the verification process.

Polyphasic Identification

- The concept of polyphasic taxonomy refers to assembly and use of many levels of information (e.g., microbial characterization, phenotypic and genotypic data, and origin of the microorganisms) can be successfully applied to microbial identification.

Let’s just agree that...

....Microbial Identification is a critical activity to ensure that objectionable microorganisms are not present in our drug products

<1117> Microbiological Best Laboratory Practices

USP <1117>Best Microbiological Lab Practices

- Aseptic Technique NEW!
- Control of Media
- Control of Test Strains
- Control of Equipment
- Lab Lay-out and Operations
- Sample Handling NEW!
- Microbiological Media Incubation Times NEW!
- Training of Staff
- Laboratory Resources NEW!
- Control of Data and Documentation
- Interpretation of Results

USP. 2010.
Second Supplement

Media Preparation

- Culture media central to quality of work in a microbiology lab
- Manufacturer's CoA
- Water – 2010 revision strengthens caution about quality of water
- Weighing of components - 2010 revision cross references USP <1251> Weighing on an Analytical Balance
- Do not overheat
- Solid media should not be re-melted more than once
- Clean glassware

Media Storage

- Transport of Prepared media a concern
  - Agar susceptible to freezing
- Molten agar should not be stored more than 8 hours
- Media should be labeled
  - Batch or lot numbers
  - Preparation and expiry dates
  - Name of media

Quality Control Testing of Media

- All media must have GP
  - General requirements
  - Specific requirements - MLT & Sterility Tests
  - pH (2010 revision cites <791> for guidance)
  - 2010 revision strengthens caution that any batch of media that fails GP cannot be used in a test

Maintenance of Microbiological Cultures

- Confirm ID of culture from culture collection before use
- Resuscitate cultures as per manufacturer's instructions
- Use a seed lot technique, do not enter a master vial more than once or refreeze stock
- Track number of passages ("Any form of subculturing is considered to be a transfer/passage").

Lab Layout and Operations

- Lab area should have separate “clean” and “live culture” areas with different access points, barriers
- Isolator technology may be useful for enhanced protection
- When a sample has growth, all further work in the “live culture” area

Sample Handling

- Critical Parameters
  - Product (or sample) composition
  - Container composition (i.e., polypropylene)
  - Time of storage (i.e., holiday weekend)
  - Temperature of storage (Room temp or Refrigerator)
  - Mixing requirements

Microbiological Media Incubation Times

- Incubation times for microbiological tests less than 3 days duration should be expressed in hours
  - *i.e.* incubate at 30 to 35°C for 18 to 72 hours.
  - QC note: incubate for the minimum specified time during growth promotion and suitability testing

Training of Personnel

- Microbiologists in pharmaceutical support lab
  - Should have academic training in microbiology or allied health sciences
  - Be trained in relevant SOPs

- Supervisors

  Should have appropriate education and training

  Managerial training should include “supervisory skills, budgeting, investigational skills, technical report writing, and relevant SOPs.”

Laboratory Resources

"The laboratory management is responsible for ensuring that the laboratory has sufficient resources to meet the existing testing requirements."

"The period of time between sample submission and initiation of testing should be tracked, as well as the period of time between end-of-test and report release (or test closure) should be tracked. Significant delays in these measures are also indications of an under-resourced laboratory staff."

Documentation

At a minimum: (cGMP)

- Microbiologist training and verification of proficiency
- Equipment validation, calibration, and maintenance
- Equipment performance during the test
- Media inventory and control
- Critical components or test performed as per procedure
- Data and calculations verified
- Reports reviewed by QAU or responsible manager
- Investigation of OOS

Maintenance of Analytical Results

- Lab write-up should include:
  - Date
  - Material
  - Microbiologist's name
  - Procedure number
  - Document test results
  - Deviations (if any)
  - Parameters of test (equip., stock cultures, media used in test)
  - Management review signature

Interpretation of Assay Results (1)

- Investigation into non-conforming results generally shows one of two reasons:
  - Lab error
  - Product failure

Interpretation of Assay Results (2)

Microbiological data can be difficult to interpret:

- Human microflora are ubiquitous in lab and production
- Microbes may not be homogenously distributed within a sample or an environment
- Sampling errors are a great concern
- Recovery of CFU are subject to considerable variability - may be around +/- 0.5 $\log_{10}$ unit.

Therefore, minor differences from the expected outcome of a test may not be significant

Interpretation of Assay Results (3)

- Corrective Action Plan
  - Needed if lab error found
  - Efficacy of corrective action should be monitored and documented
- Invalidated Test

  Invalidation of a test due to attributable error must be documented
- Confirmatory Testing (retesting)
  - SOP should be in place to describe conditions for confirmatory testing of results. Should not “test product into compliance”.

For Your Information...

...I'm finished

Thank You