Sterility Testing and USP: What you need to know

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Disclosure

- I have no relative financial relationships to disclose related to this presentation

Objectives

- Identify the 2 compendia approved sterility tests
- Recognize when sterility testing is required per USP<797>
- Determine who should perform sterility testing
- Know what a growth promotion test is

Disclaimer

- I am speaking in an individual capacity and not as a representative of any organization or committee regardless of my status, membership or affiliations with any professional entity.
- The views and opinions presented are entirely their own.
- The opinions presented do not necessarily reflect the views of any organization I may be associated with, nor should they be construed as an “official” explanation or interpretation of any USP chapter or any State Board of Pharmacy rule/law.

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Overview

- Compounding History
- Issues/Problems
- What is Sterility Testing?
- Why Sterility Test?
- USP 71
- How much to sample?
- With what do we sample?
- Method Suitability
- Membrane Filtration
- Direct Inoculation
- Process Validation
- Probability Testing
- Results Evaluation
- Limitations of Testing
- Beyond Use Dating

Compounding History

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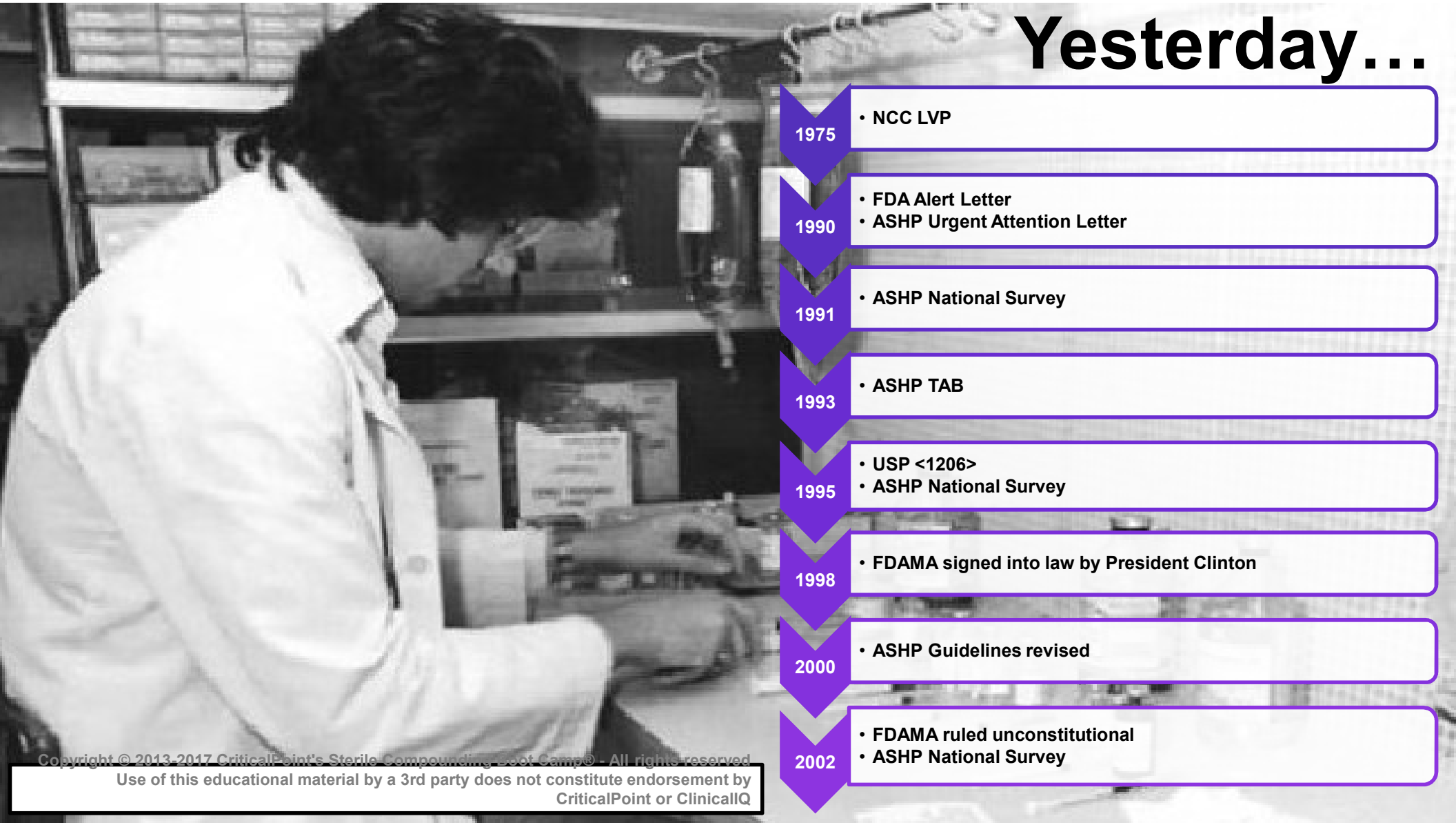
History of Compounding

- All states license pharmacists to compound
  - States laws vs. Federal laws (FDA)
  - The federal government, through the FDA, is arguing that patient safety is in jeopardy
- Each state has varying degrees of regulations and oversight and enforcement of compounding practices
  - Only 31 states require direct compliance with USP 797 after 12 years
- Until USP <797>, no consistent and enforceable compounding standard of practice existed

History of Sterile Compounding

- Despite the chapter's uniform sterile compounding standards, schools of pharmacy may not always include sterile compounding
- Only 1 in 6 pharmacy graduates are prepared for sterile compounding work*
- In 2014, less than 10 schools of pharmacy conducted training in actual cleanroom settings

*Hellums M, Alverson SP, Monk-Tutor MR. Instruction on compounded sterile preparations at U.S. schools of pharmacy. AJHP. Volume 64, Nov 1, 2007: 2267-74.



Yesterday…

1975
- NCC LVP

1990
- FDA Alert Letter
- ASHP Urgent Attention Letter

1991
- ASHP National Survey

1993
- ASHP TAB

1995
- USP <1206>
- ASHP National Survey

1998
- FDAMA signed into law by President Clinton

2000
- ASHP Guidelines revised

2002
- FDAMA ruled unconstitutional
- ASHP National Survey

Today…

2004
- January 1, USP 797 first published

2004
- November, NIOSH Alert published

2008
- USP Chapter <797> revised, new standard effective June 2008

2011
- 1st CriticalPoint 797 Compliance Survey

2012
- CDC and CMS recognizes USP Chapter <797>
- NECC tragedy
- 2nd USP 797 Compliance Survey

2013
- 3rd USP Chapter <797> Compliance Study
- The Drug Quality and Security Act becomes law

2015
- FDA Guidance to 503B Pharmacies
- 28 States require compliance with USP 797

Issues/Problems

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Who's been involved in compounding contaminated drugs?

Retail/Community-based

Homecare/Home Infusion

Hospital

Commercial outsourcer

What do these entities have in common?

- are licensed as pharmacies by their respective state boards of pharmacy
- considered compounding pharmacies by the FDA
- represent the full spectrum of practice settings run by pharmacists

What's the damage?

Since 2001 ➡ over 25 pharmacy compounding incidents with 1,049 adverse events, including 89 deaths, have been reported.

- Contamination of sterile preparations was the most common compounding error, though others were the result of pharmacists' and technicians' miscalculations and mistakes in filling prescriptions.

Why Sterility is Important?

- Facilitates early detection of contamination and its source which may include:
  - Personnel
  - Work surfaces
  - Supplies
  - Equipment
  - Failure of engineering controls

Courtesy CBS News

This is a image of the fungus growing from a sample taken from a patient's spinal fluid.

New England Compounding Center (NECC) Meningitis Outbreak	
Date	September 21, 2012– October 23, 2013 (no further CDC updates expected)
Location	USA (20 States)
Cause	Fungal meningitis contamination of steroid medication
Injuries	751 total case count; 384 meningitis and spinal infection; 7 stroke; 325 paraspinal/spinal infection; 33 peripheral joint infection; 2 spinal and peripheral joint Some patients recovering from the meningitis are falling ill again. Sufferers of the new infection are now coping with epidural abscesses and infections near the injection site.
Death(s)	64
Litigation	More than 20 lawsuits filed against NECC

The scale of the meningitis outbreak makes this event the worst among a series of fatal or harmful infections and overdoses linked to pharmacy compounding practices in the US rivaling other key drug safety issues in the past that have led to substantial drug safety legislation.

Year	State	Description
2013	Connecticut	FDA announced that a compounding pharmacy in New Jersey was voluntarily recalling all of its products after a Connecticut hospital reported that 5 bags of magnesium sulfate from the pharmacy were contaminated with mold. The pharmacy has since been closed by the NJ Division of Consumer Affairs
	Georgia, Louisiana, South Carolina and Indiana	A compounding pharmacy in Augusta, Georgia, is voluntarily recalling 79 lots of bevacizumab-filled syringes (Avastin, Genentech) intended for retinal injections because of the risk for eye infection, the US Food and Drug Administration (FDA) announced yesterday.
	Texas	A batch of compounded IV Calcium Gluconate found to be contaminated with Rhondococcus equii. 15 infected patients, 2 deaths (relationship to drug not known)
2014	Oregon	A patient died after being given rocuronium instead of fosphenytoin. The order was correct, the bag was labeled correctly, it was independently checked by a 2nd person however the rocuronium vials were not noticed and the error went undiscovered.

What is Sterility Testing?

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What is “Sterility”?

- “Free from bacteria or other microorganisms”
  - American Heritage's Definition of Sterility
- “Within the strictest definition of sterility, a specimen would be deemed sterile only when there is complete absence of viable microorganisms from it.”
  - <1211> *Sterilization and Sterility Assurance of Compendial Articles*
- Is it possible to demonstrate complete absence of microorganisms from a CSP?
- Absolute sterility can’t be demonstrated without the complete destruction of every article from the lot of CSPs.

Sterility Testing

- Only required when USP <797> BUD limits are exceeded or when more than 25 units of HR CSPs are prepared.
- NO testing is required for any risk-level CSP prepared if handled within the limits, terms and conditions described in USP Chapter <797>

Standards of Sterility

- USP Chapter <1> Injections
  - Parenteral articles are prepared….to ensure they meet pharmacopeial requirements for sterility, pyrogens,...
  - Sterility Tests – Preparations for injection meet the requirements under Sterility Tests <71>

Standards of Sterility

- Aseptic Processing from USP Chapter <1211>:
  - “While there is general agreement that sterilization of the final filled container as a dosage form or final packaged device is the preferred process for assuring the minimal risk of microbial contamination in a lot, there is a substantial class of products that are not terminally sterilized but are prepared by a series of aseptic steps.”

Standards of Sterility

- USP <71> Sterility Tests states:

  “These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by method suitability of the sterilization process or of the aseptic processing procedure.”

Why Sterility Test?

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Why do we perform Sterility Tests?

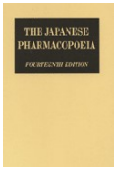
Because the GMP's says so!

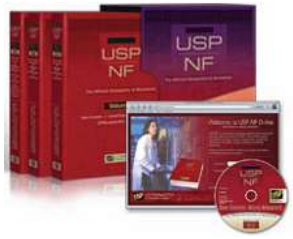
21CFR Part 211§167 (a)

- ***"For each batch of drug product purporting be sterile and/or pyrogen-free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."***

Regulations... Pharmacopeias... Guidance

- Pharmacopeias
  - USP Chapter <71>
- Other Guidance Agencies
  - FDA : Food and Drug Administration
  - PDA : Parenteral Drug Administration
  - ISO : International Organization for Standardization





USP Chapter <71>

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Points to Consider from USP <71>

- Provides details on Media, Microorganisms, Test Conditions, Sample Considerations, Suitability (aka Validation) and Methodologies

- "*The test may be carried out using the technique of Membrane Filtration or by Direct Inoculation of the Culture Medium with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits; that is, for filterable aqueous preparations, for alcoholic or oily preparations, and for preparations miscible with, or soluble in, aqueous or oily solvents, provided these solvents do not have an antimicrobial effect in the conditions of the test.*"

- "*Use membrane filters having a nominal pore size not greater than 0.45 µm, in which the effectiveness to retain microorganisms has been established*"

USP <71>

Points to Consider from USP <71>

- "These Pharmacopeial procedures are NOT by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedures" – USP <71>
  - Not intended as a sole product release test (See USP <1211>)
  - Represents one set of data which contributes to the decision of whether or not the product lot meets the stated claims
  - Pass/Fail Destructive test
  - Not for viruses, mycoplasma or endotoxin
  - Long incubation period : 14 calendar days (Harmonized in 1998)
  - Probability-based
  - Not statistically valid, not representative

Points to Consider from USP <71>

- Lot Homogeneity : A *lot* is defined as homogeneous closed set, prepared in such a way that the risk of contamination is the same for each of the component units of this lot = Manufacturing Goal
- The probability to discover microorganisms increases with their number in the sample and varies according to the capacity of growth of the different types

How much to sample?

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Products to be Examined & Sample Considerations

- Number of products needed for testing is based upon several Factors:

1. How the Product is prepared
2. Type of Product
3. Batch Size

Number Items in Batch	Minimum Number Items to be Tested for Each Medium (unless otherwise justified and authorized)*
Parenteral Preparations	
• Not more than 100 containers	10% or 4 containers, whichever is the greater
• More than 100 but not more than 500	10 containers
• More than 500 containers	2% or 20 containers, whichever is less
For large-volume parenterals	2% or 10 containers, whichever is less
Antibiotic solids	
• Pharmacy bulk packages (< 5g)	20 containers
• Pharmacy bulk packages (≥ 5g)	6 containers
Bulks and blends	See bulk solid products
Opthalmic and other noninjectable preparations	
• Not more than 200 containers	5% or 2 containers, whichever is the greater
• More than 200 containers	10 containers
If the product is presented in the form of single dose containers, apply the scheme shown above for preparations for parenteral use.	
Devices:	
Catgut and other surgical sutures for veterinary use	2% or 5 packages, whichever is the greater up to a maximum of 20 packages
• Not more than 100 articles	10% or 4 articles, whichever is the greater
• More than 100, but not more than 500 articles	10 articles
• More than 500 articles	2% or 20 articles, whichever is less
Bulk Solid Products	
• Up to 4 containers	Each container
• More than 4 containers, but not more than 50 containers	20% or 4 containers, whichever is greater
• More than 50 containers	2% or 10 containers, whichever is greater

*refer to USP <71> for additional information

Quantity Per Container	Minimum Quantity to be Used (unless otherwise justified and authorized)*
Liquids (other than antibiotics)	
• Less than 1 mL	The whole contents of each container
• 1 – 40 mL	Half the contents of each container, but not less than 1 mL
• Greater than 40 mL and not greater than 100 mL	20 mL
• Greater than 100 mL	10% of the contents of the container, but not less than 20 mL
Antibiotic liquids	1 mL
Other preparations soluble in water or in isopropyl myristate	The whole contents of each container to provide not less than 200 mg
Insoluble preparations, creams, and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
• Less than 50 mg	The whole contents of the container
• 50 mg or more, but less than 300 mg	Half the contents of each container, but not less than 50 mg
• 300 mg – 5 g	150 mg
• Greater than 5 g	500 mg
Devices	
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30 cm long)
Surgical dressing/cotton/gauze (in packages)	100 mg per package
Sutures and other individually packaged single-use material	The whole device
Other medical devices	The whole device, but into pieces or disassembled

*refer to USP <71> for additional information

Product Requirements – Minimum Quantity per Unit for Each Medium

Liquids	Minimum Quantity
< 1 mL	The whole contents of each container
1-40 mL	Half the contents of each container, but not less than 1 mL
>40 - <100 mL	20 mL per container
>100 mL	10% of the contents of the container, but not less than 20 mL
Antibiotic Liquids	1 mL

Product Requirements – Minimum Quantity per Unit for Each Medium

Solids	Minimum Quantity
< 50 mg	The whole contents of each container
50 - <300 mg	Half the contents of each container, but not less than 50 mg
300 mg – 5 g	150 mg per container
>5 g	500 mg per container

Insoluble preparations, creams, and ointments to be suspended or emulsified - Use the contents of each container to provide not less than 200 mg

Example 1 - 84 units of 10 mL

- 10 ml is between 1 and 40 mL – Table 2 tells us to use ½ volume or 5 mL per unit
- 84 units is less than 100 – Table 3 tells us to use the greater of 4 units or 10% of the batch = 8 units per medium
- We therefore must test at least 5 mL from each of 8 units for each medium

Example 2 – 6 units of 1.5 mL

- 1.5 ml is between 1 and 40 mL – Table 2 tells us to use ½ volume but not less than 1 mL – therefore we test 1 mL mL per unit
- 6 units is less than 100 – Table 3 tells us to use the greater of 4 units or 10% of the batch = 4 units per medium
- We therefore must test at least 1 mL from each of 4 units for each medium.

With what do we sample?

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Sterility Media & Rinse Solutions

- Pharmacopeial Medium
  - Soybean Casein Digest Broth (SCDB, TSB)
  - Fluid Thioglycollate Medium (FTM)
  - Clear Fluid Thioglycollate Media (CTM)

- Pharmacopeial Rinse Solutions
  - Fluid A, D and K

Culture Media - TSB

SCDB – Soybean Casein Digest Broth
TSB – Tryptic Soy Broth, Trypticase Soy Broth, Tryptone Soy Broth

- pH of 7.3 ± 0.2 after Sterilization
- Store at 2º - 25ºC in a sterile well-closed container, unless it is intended for immediate use.
- Do not use the medium for a longer storage period than has been validated

Soybean–Casein Digest Medium

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean Meal	3.0 g
Sodium Chloride	5.0 g
Dibasic Potassium Phosphate	2.5 g
Dextrose Monohydrate/Anhydrous	2.5/2.3 g
Purified Water	1000 mL

Culture Media - FTM

FTM is to be incubated at 33.5 ± 2.5ºC unless used instead of Soybean–Casein Digest Medium provided that it has been validated as described in Growth Promotion Test of Aerobes, Anaerobes and Fungi.

- pH of 7.1 ± 0.2 after Sterilization
- Store at 2º - 25º in a sterile well-closed container, unless it is intended for immediate use
- If more than the upper 1/3rd of the container is pink, medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and by cooling quickly, taking care to prevent the introduction of non-sterile air into the container.
- Do not use the medium for a longer storage period than has been validated

Fluid Thioglycollate Medium

L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose Monohydrate/Anhydrous	5.5/5.0 g
Agar	0.75 g
Yeast Extract (water-soluble)	5.0 g
Pancreatic Digest of Casein	15.0 g
Sodium Thioglycollate	0.5 g
or Thioglycolic Acid	0.3 mL
Resazurin Sodium Solution (1 in 1000), freshly prepared	1.0 mL
Purified Water	1000 mL

Culture Media - CTM

Fluid Thioglycollate Medium

L-Cystine	0.5 g
Sodium Chloride	2.5 g
Dextrose Monohydrate/Anhydrous	5.5/5.0 g
Agar	0.75 g
Yeast Extract (water-soluble)	5.0 g
Pancreatic Digest of Casein	15.0 g
Sodium Thioglycollate	0.5 g
or Thioglycolic Acid	0.3 mL
Resazurin Sodium Solution (1 in 1000), freshly prepared	1.0 mL
Purified Water	1000 mL

- Alternative Fluid Thioglycollate (aka CTM) follows the same recipe as FTM
- Prepare a mixture having the same composition as that of the Fluid Thioglycollate Medium, but omitting the agar and the resazurin sodium solution. Sterilize as directed above. The pH after sterilization is 7.1 ± 0.2. Heat in a water bath prior to use and incubate at 30–35 under anaerobic conditions.
- Different formulas for each vendor

Culture Media
Regulatory Requirements : USP

- Other Media:
  - Equivalent commercial media may be used provided that they comply with the growth promotion test.
  - Products containing mercurial preservative that cannot be test by membrane filtration method : FTM at 20-25°C may be used instead of TSB Validation is required
  - Where prescribed or justified and authorized alternative FTM may be used (without agar and resazurin sodium solution).
  - For Direct Innoculation : Media for Penicillins or Cephalosporins. (SCDB and FTM supplemented with β lactamase)

Culture Media Regulatory Requirements : USP

- Sterility : No growth after 14 days
- Growth Promotion Test of aerobes, anaerobes and fungi
  - SCDB/TSB: *Aspergillus brasiliensis , Bacillus subtilis, Candida albicans*
  - FTM:*Clostridium sporogenes* or *Bacteroides vulgaris*, *Pseudomonas aeruginosa* or *Micrococcus luteus (Kocuria rhizophila), Staphylococcus aureus*
  - Alternative FTM : *Clostridium sporogenes*
  - Not more than 100 CFU in separate portion of medium
  - Strains used for inoculation are not more than 5 passages removed from the original master seed-lot – This includes any EM Strains
  - Not more than 3 days in case of bacteria
  - Not more than 5 days in case of fungi
  - The media is suitable if visible growth of the micro-organisms occurs
  - Each batch of media is tested (ready-prepared or dehydrated media)
    - Per supplier, per lot number, per preparation, per shipment

Avoid false positive

Avoid false negative

Culture Media Regulatory Requirements : USP

- Suitability Tests
  - The suitability test have to be carried out before, or in parallel, with the test on the product to be examined
  - Sterility
    - Pharmacopoeias require that sterility testing should be performed to confirm the sterility of the microbiological medium.
  - Growth promotion Test
    - To confirm the ability of the test medium to support the growth and reproduction of selected microorganisms.
    - Test each lot of ready-prepared medium and each batch of medium prepared either from dehydrated medium or from ingredients
    - Per supplier, per lot number, per preparation

Sterile Buffers – Sterility Test

- Purpose
  - Assure the sterility of rinsing fluids
  - *Prevent the occurrence of false positive results* during the sterility test of the test article
- The test method used : membrane filtration
- The rinse buffer is considered sterile if after filtration no microbial growth (visually determined by lack of turbidity in the media) is observed within the incubation period of 14 days.
- Concurrent negative control with Sterility test of product

Sterile diluent buffers Regulatory Requirements : USP

- USP
  - called diluting and rinsing fluids
  - three types: fluid A, D and K
  - Buffer for penicillin or cephalosporin (FA + β lactamase)

Type	Characteristics	Application
Fluid A (USP) / neutral solution of meat or casein peptone (EP)	0.1% Peptone: source of Carbon & Nitrogen pH 7.1 ± 0.2* maintained osmotic equilibrium (pH prior to sterilization)	▪ Suitable as a general rinse buffer ▪ Works well with most samples ▪ Excellent to dissolve or dilute samples ▪ Excellent to reconstitute commercial microorganisms ▪ Excellent transport medium for microorganisms
Fluid D (USP)	**1 l Fluid A + 1 ml polysorbate 80 (0,1%)** Polysorbate 80: will neutralize some preservatives Peptone: source of Carbon & Nitrogen pH 7.1 ± 0.2* maintained osmotic equilibrium (* prior to sterilization)	▪ Suitable for testing specimens that contain lecithin or oil ▪ Excellent for rinsing sterile pathways of devices ▪ Works well with most antibiotics ▪ Needed for rinse method testing of Medical Devices
Fluid K (USP) (neutral solution with emulsifying agent (EP))	Beef extract and peptone: provide nutrients for recovery of injured and fastidious microorganisms **Polysorbate 80 at a concentration of 10 g/l (1 %)** Polysorbate 80 : will neutralize some preservatives pH 6.9 ± 0.2 maintained osmotic equilibrium (* prior to sterilization)	▪ Suitable for testing specimens that contain petrolatum ▪ Suitable for oils and oily solutions ▪ Excellent for rinsing pathways of Medical Devices ▪ Good for “difficult” sample to filter or to dissolve samples

Method Suitability

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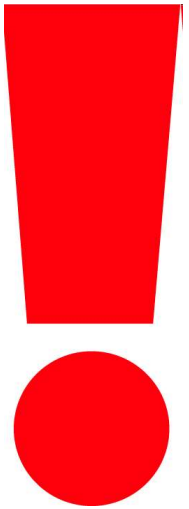
Method Suitability Test

- Can we neutralize any antimicrobial properties of the medication?
  - Use specified challenge organisms
  - Use specified total amounts of products

Method Development

- The method for performing the Sterility Test must be confirmed before Method Suitability (a.k.a Validation)
  - Filterability
  - Chemical Compatibility
  - Rinsing Fluids & Volumes
  - Potential Inhibition issues
  - Membrane Compatibility
  - Quantity of Samples to be tested



Data for removal of certain bacterial requirement will be figured here – This data can be used to petition to the regulatory agencies for dismissal of this organism

Culture Media Regulatory Requirements : USP

- Growth Promotion Test : Microorganisms

Table 1. Strains of the Test Microorganisms Suitable for Use in the Growth Promotion Test and the Method Suitability Test

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
Pseudomonas aeruginosa[1]	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium	
Clostridium sporogenes[2]	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
Aspergillus brasiliensis (Aspergillus Niger)	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

[1] An alternative microorganism is *Kocuria rhizophila (Micrococcus luteus)* ATCC 9341.

[2] An alternative to *Clostridium sporogenes*, when a nonspore-forming microorganism is desired, is *Bacteroides vulgatus* (ATCC 8482).

Method Suitability Test for Each Challenge Organism

- Filter maximal amount of medication to be tested
- Filter 2 volumes (100 mL?) of diluting fluid
- Add third volume, inoculate with <100 CFU challenge organism
- Filter
- Show microbial growth from filter in relevant medium at relevant temperature in 5 days

Method Suitability – Examples

- Example 1 - 84 units of 10 mL
  - 5 mL from 8 units = 40 mL for each organism; there are 6
  - 6 x 40 mL = 240 mL total for Method Suitability

- Example 2 – 6 units of 1.5 mL
  - 1 mL from 4 units = 4 mL for each organism
  - 6 x 4 mL = 24 mL total for Method Suitability

Method Suitability – Membrane Filtration or Direct Inoculation

- Previously known as the Bacteriostasis & Fungistasis (B&F) Test
- Using the method that was developed, Method Suitability is performed to show that your product conforms with USP <71>
  - Membrane Filtration or Direct Inoculation

Method Suitability: Objectives

- Certain products may contain bacteriostatic or fungistatic agents which if not neutralized, will inhibit the growth of viable microorganisms present in the product, producing false negative results.
- Neutralization of these products may be achieved via
  - dilution
  - Chemical neutralization
  - Filtration & Rinsing
  - Enzyme activity
- Combination of the above four methods

Method Suitability: Objectives

The B&F test confirms that the neutralization method will prevent the B & F properties of the test article (neutralizer **efficacy**) without sacrificing recovery of viable microorganisms (neutralizer **toxicity**).

This prevents the occurrence of false negative results

They have to be applied to :

Test methodology
- Direct inoculation
- Membrane filtration

- Devices
  - Open funnel

- Packaging integrity (isolator) :
  - Fluids (media, rinsing fluids)
  - Devices

- Articles to be tested

Method Suitability: Objectives

- This suitability test (B&F) is performed
  - When the test for sterility has to be carried out on a new product
  - Whenever there is a change in the experimental conditions or process change that could effect the parameters of the test

- The B&F test may be performed simultaneously with the Test for Sterility of the product to be examined
  - ....but before the results of this test are being interpreted

Method Suitability Test: Regulatory Requirements

Growth Promotion Test as a positive control

- If clearly visible growth, visually comparable to that in the control vessel without product:
  - The test may then be performed without further modification
- If no visible growth, visually comparable to that in the control vessel without product:
  - Modify the conditions in order to eliminate the antimicrobial activity and repeat the method suitability test
- "If the product has antimicrobial properties, wash the membrane not less than 3 times by filtering through it each time the volume of the chosen sterile diluent used in the validation test. Do not exceed a washing cycle of 5 times 100 ml, even if during validation it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity." – USP <71>

Avoid false negative

Types of USP 71 Sterility Test

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Sterility Testing

- Two separate tests
  - Membrane Filtration
  - Direct Transfer
- 2 media & temperatures
- Requires Growth
  - Incubation period - 14 days

Moldenhauer, J and S. Sutton. 2004. Towards an Improved Sterility Test. PDA J of Science and Technology 58(6):284-286.

Membrane Filtration

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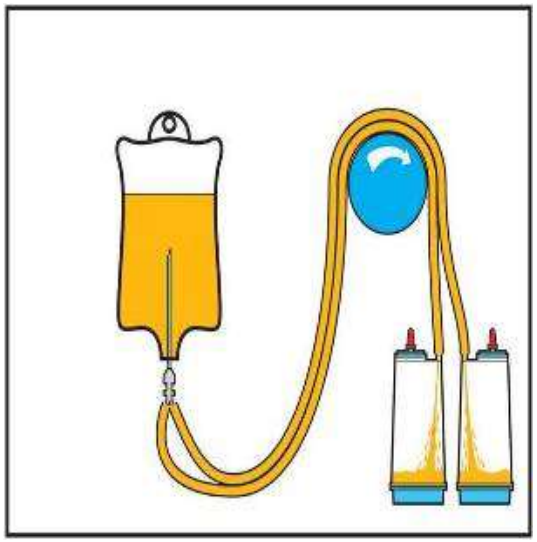
Sterility Testing (Membrane Filtration)



Millipore Equinox Steritest System

Membrane Filtration

- Filter required amount of product through two filters
- Neutralize/Rinse
  - 3 100 mL volumes suggested
  - Formulations for dilution fluids suggested
- One filter into Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25^{o}C for 14 days
- One filter into Fluid Thioglycollate Medium (FTM) – incubate at 30-35^{o}C for 14 days

Membrane Filtration

- Perform the test with test article using exactly the same methods as for the sterility test :
  - Pre-Wet the membrane(s) with the appropriate rinse buffer
  - Transfer the test article to the membrane
  - Rinse membranes with the appropriate rinse buffer
- Inoculate final rinse buffer with specified test microorganism*
  - *The same as for the Growth Promotion Test
  - Not more than 100 CFU
- Add media and incubate (repeat for 2nd media)
  - For not more than 3 days for bacteria and 5 days for fungi
- Examine for positive growth vs a control without the product (positive control)

Direct Inoculation

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Direct Inoculation

- Place required amount of product into sufficient recovery medium (with neutralizers?)
  - Soybean Casein Digest Broth (SCDB or TSB) – incubate at 20-25^{o}C for 14 days
  - Fluid Thioglycollate Medium (FTM) – incubate at 30-35^{o}C for 14 days

Direct Inoculation

- Perform the test with test article using exactly the same methods as for the sterility test :
  - Add sterile vented needle through septum (if applicable) & remove cap of media (repeat for 2nd media)
- Discard the vented needle
- Aseptically transfer the test article to the appropriate media
- Inoculate the media with specified test microorganism*
  - *The same as for the Growth Promotion Test
  - Not more than 100 CFU
- Cap media and add a sterile vented needle through septum (if applicable)
- Repeat for second media and incubate both bottles
- Examine for positive growth vs. a control without the product (positive control)

Process Validation

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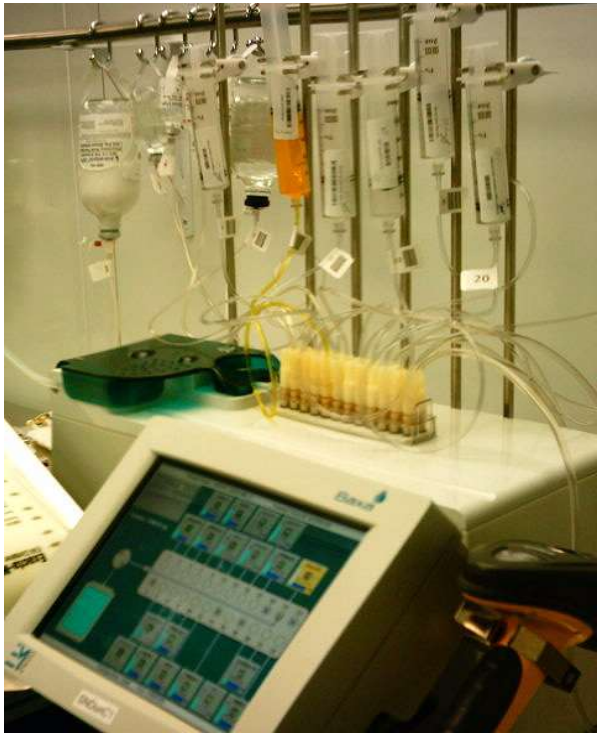
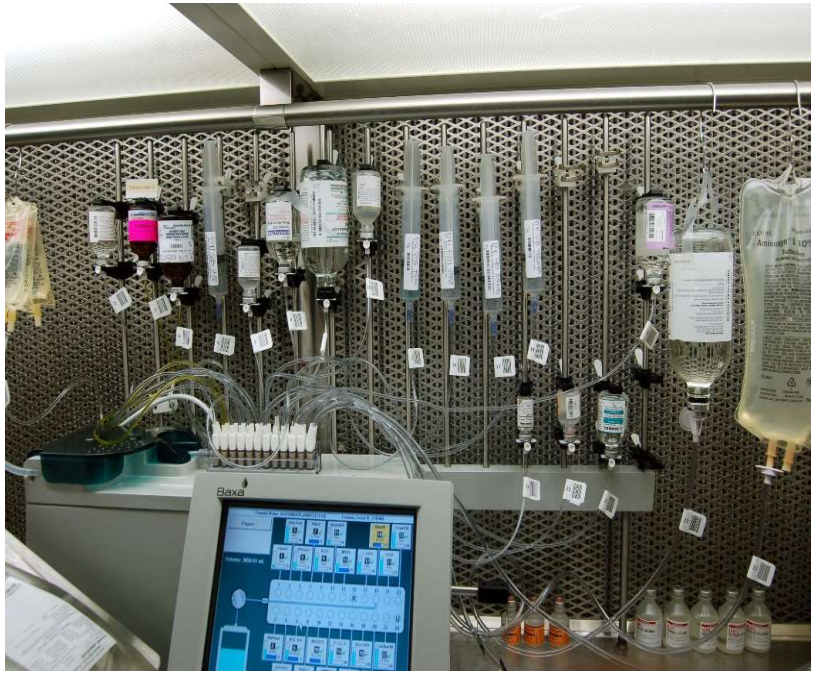
Process Verification

- USP <71> Sterility Tests states:
  - “These Pharmacopeial procedures are not by themselves designed to ensure that a batch of product is sterile or has been sterilized. This is accomplished primarily by validation of the sterilization process or of the aseptic processing procedure”



Process Verification (continued)

- A critical quality metric that demonstrates the operating capability of a process
  - What evidence can be produced to “prove” that a TPN bag can be aseptically compounded on an ACD?➡ Process verification procedure
  - Mimic compounding procedure using TSB as the components instead of macro and micro additives
  - Perform it under “worst-case” scenarios and in a robust manner

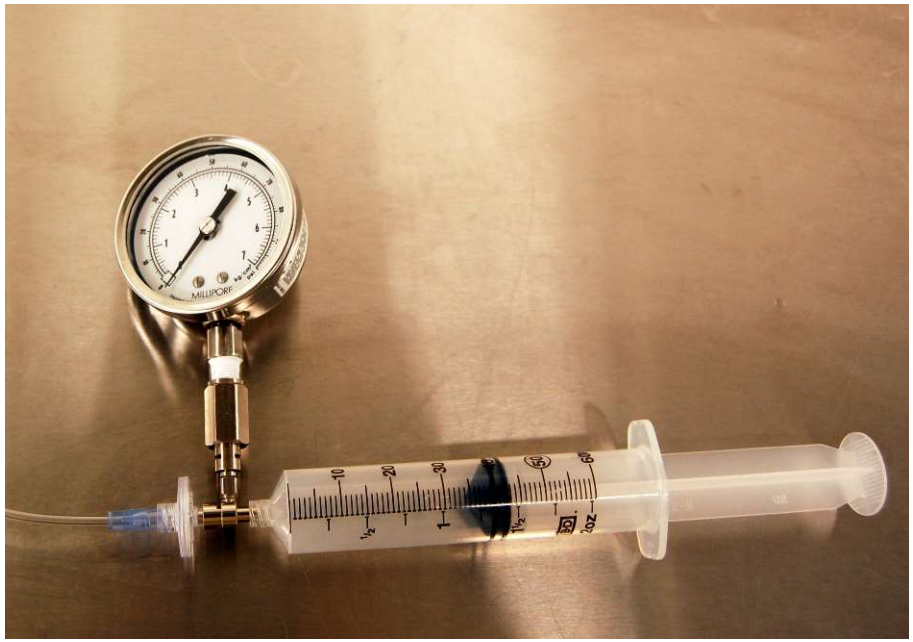


Process Verification (continued)

- The basic principles for validation may be stated as follows:
  - The process has the capability of producing and/or yielding the expected results within required/desired parameters;
  - Demonstrate that controlling, monitoring, and/or measuring equipment and instrumentation are capable of operating within the parameters prescribed for the process equipment;
  - Perform replicate cycles (runs) representing the typical compounding methods to demonstrate that the processes have been operated within the desired parameters for the process and that the yield or CSP consistently meets predetermined specifications (sterility, pyrogenicity, potency and identity); and
  - Monitor the validated process during routine operation. As needed, requalify and recertify.

Process Verification (continued)

- Processes that should be verified
  - Compounding methods
  - Equipment used in compounding methods
  - Delivery of solution volume via ACDs
  - Autoclave or Dry-Heat oven sterilization cycles
    - Use of Biological Indicators (BIs)
  - Filtration methodology
    - FDA approved/challenged filters
    - Filter integrity testing



Process Verification (continued)

- Use Tryptic Soy Broth (TSB) (or Soybean-Casein Digest Medium (SCDM)) as the source containers
- Simulate the compounding of CSPs
- Ideally, should verify all compounding processes
  - SVPs
  - Intrathecal
  - Batch preparation
- Incubation of compounded media fill x 14 days
- 14 days @ 25-35˚C or 7 days @ 30-35˚C followed by 7 days @ 25˚C

Probability Testing

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Probability of Sterility

- “The sterility of a lot purported to be sterile is therefore defined in probabilistic terms, where the likelihood of a contaminated unit or article is acceptably remote.”

Process Simulation Testing (PST) Limitations

- It is only a point-in-time representation of the:
  - Environment
  - Equipment
  - Procedure
  - Personnel involved
- Test Media for Suitability
  - Sterility
  - Growth Promotion
    - After the incubation period is complete
- USP Chapter <797> USP 36- NF 31.
  - Does not recognize PST as a means of extending BUD

Critical Concepts of Sterilization

- Sterility Assurance Level (SAL) is the probability of a non-sterile item making it through the validated sterilization process.
- Items terminally sterilized by moist or dry heat, irradiation, or chemical sterilants have a SAL of 10^{-6}
  - 1 nonsterile item per 1 million items sterilized
- Items prepared aseptically with a 0.22 micron filter have a SAL of 10^{-3}
  - 1 nonsterile item per 1 thousand items sterilized

Probability Testing

Table 1: The Relationship between the Probability of Passing the First and Repeat Sterility Tests and the Percentage of Nonsterile Units in the Lot Contamination Rate or Percentage of Nonsterile Units in a Batch						
	0.1	1	5	10	20	50
Probability of passing the sterility test, n = 20	0.98	0.82	0.36	0.12	0.012	<0.00001
Probability of passing the repeat sterility test, n = 20	0.99	0.99	0.84	0.58	0.11	0.002

Cundell AM. "Review of the Media Selection and Incubation Conditions for the Compendial Sterility and Microbial Limit Tests," *Pharm.Forum* **28** (6), 2034–2041.

Results Evaluations

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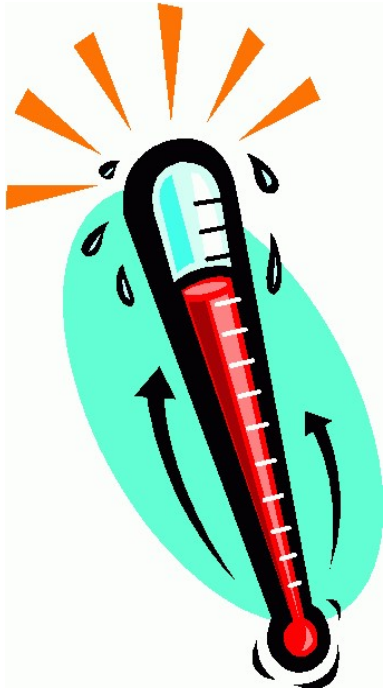
Incubation Specifications

- Current Test Conditions
  - 14 calendar days
- Harmonization
  - 14 Days Incubation for all products
  - Compensate for sub-optimal Growth
  - Account for inherent slow growers
  - Repair injured cells
  - Questionable turbid reaction after 14 days
    - Subculture at least 4 additional days
- Examination post 14 days not advisable
- Verify slow growers from EM data

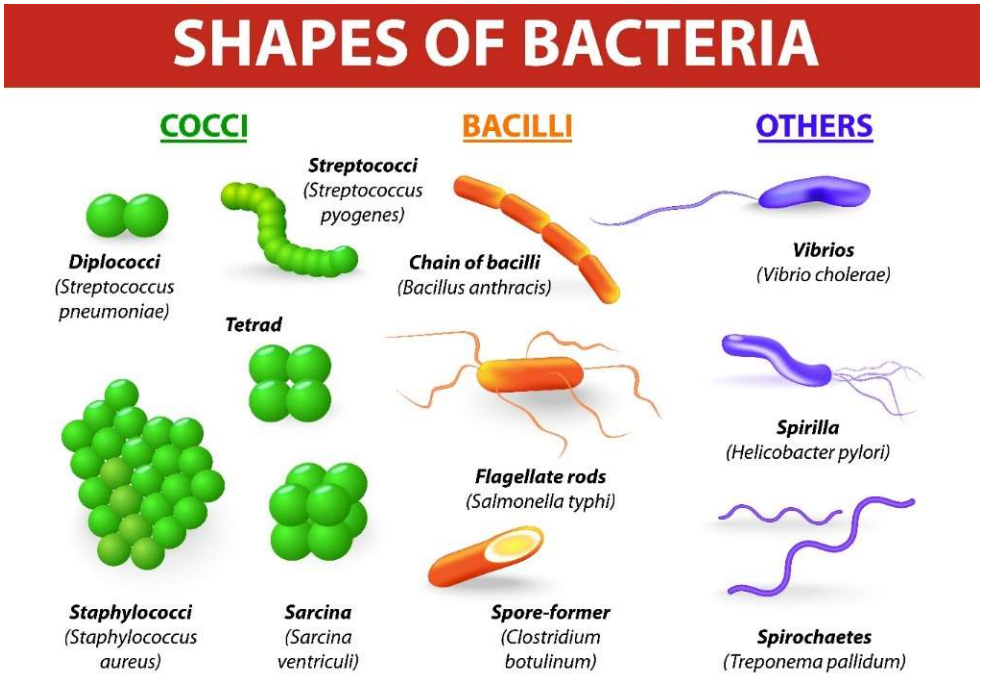


Speciation to Genus Level Currently Required

- USP Chapter <797> USP 34-NF 29

"Regardless of the number of cfu identified in the pharmacy, further corrective actions will be dictated by the identification of micro-organisms recovered (at least the genus level) by an appropriate credentialed laboratory of any microbial bioburden…"

Proposed Chapter requires that when samples exceed the action levels, genus must be identified and if possible, the species of microorganisms must be identified with the assistance of a credentialed microbiology laboratory



Interpretation of Results

- Operator training
  - “Training curricula should be established for each laboratory staff member specific for their job function. They should not independently conduct a microbial test until they are qualified to run a test” (USP, <1117>)
  - Personnel should undergo periodic recertification
- Qualification process
  - Visual test?
  - Readout of media fills?
  - Start with negative controls?

Interpretation of Results Regulatory Requirements : USP

Observation and Interpretation of results

- Observations at intervals during the incubation period – good practice
  - Intermediate reading (1 person)
  - Final reading (2 persons)
  - Conditions (light, background)
  - Examine the media for macroscopic evidence of microbial growth

- If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer portions (each not less than 1ml) of the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days

- If no evidence of microbial growth is found:

- If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined.

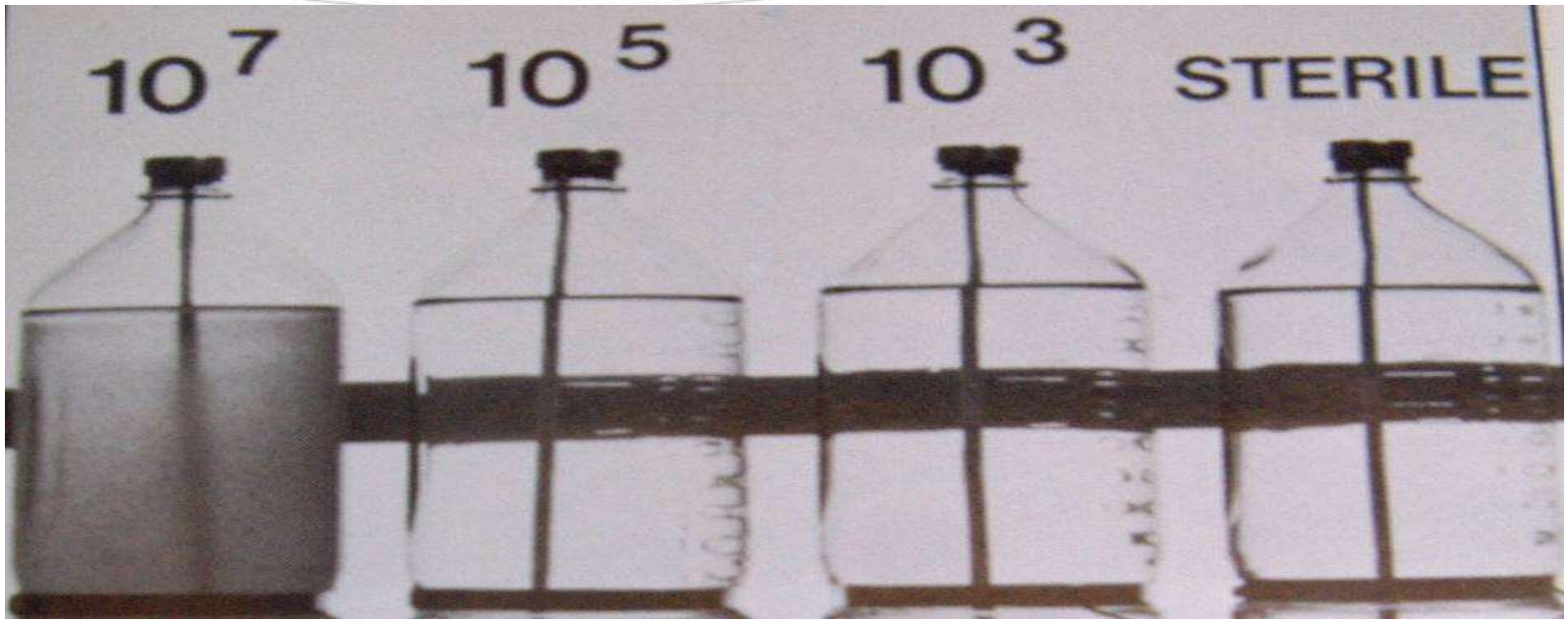
Interpretation of Results Regulatory Requirements

If the test is declared to be invalid it is repeated with the same number of units as in the original test (USP <71> / EP, 2.6.1)

If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility (USP <71> / EP, 2.6.1)

If microbial growth is found in the repeat test, the product examined does not comply with the test for sterility (USP <71> / EP, 2.6.1)

Non-Visibility of Microbial Contamination



Numbers of Bacteria per mL in 1L bottles
Millipore Corp. *Hospital Pharmacy Filtration Guide* (Cat. No. MP801)
Bedford, MA; 1980:3

End-product Evaluation

- Sterility testing required for CSPs that exceed <797> storage periods (all 3 risk levels)*
  - Comply with USP <71> standards
    - Two growth media required: TSB and FTM
    - Membrane Filtration : this is the preferred method if the CSP is filterable
    - or Direct Inoculation
  - Or another method (not in <71>) if verification results demonstrated equivalence to USP <71>

14 days of incubation is required!

Limitations of Testing

Sterility Testing: Limitations

“The probability of detecting very low levels of contamination even when it is homogeneous throughout the batch is very low.” EP7

«A satisfactory result only indicates that no contaminating micro-organism has been found in the sample examined in the condition of the test»
USP33/EP7

VALIDATE AND PERFORM THE TEST FOR STERILITY IN THE BEST CONDITIONS IN ORDER TO GET

THE MOST RELIABLE DATA

Sterility Testing

- Limitation of the Sterility Test with Respect to Sample Size
  - It should be recognized that the USP sterility test might not detect microbial contamination if present in only a small percentage of the finished articles in the batch, because the specified number of units to be taken imposes a significant statistical limitation on the utility of the test results.
  - This inherent limitation however, has to be accepted since current knowledge offers no nondestructive alternatives for ascertaining the microbiological quality of every finished article in the lot, and it is not a feasible option to increase the number of specimens significantly.

Sterility Testing

- “Passing” a sterility test does not guarantee that every unit in that batch is sterile.
- Sterility testing is required to provide extended BUD.
- The use of two types of medias is required.
- Membrane filtration is the preferred method of sterility testing.
- BUDs are not universal and must be verified by each vendor.
- Must be based on sterility testing according to USP 71 or other procedures, methods or processes that have been ***proven to be equivalent or superior with statistical significance***.

Challenge external testing labs and vendors on how they accept samples less than the quantities prescribed in USP 71, Table 3.

Who Should Conduct the Tests?

- Sterility testing shall be performed in a qualified microbiology laboratory by microbiologists.
  - Pharmacies do not have the education, qualifications or the physical facilities to do the testing.
    - This testing involves the use of live bacteria and fungi to test the ability of the media to support growth of a variety of microorganisms.
- Ideally, media used to perform a sterility test should be validated by following the Growth Promotion Tests (GPT) in USP <71>.
  - Each lot of media should have a Certificate of Performance that demonstrates that the lot meets USP compendial standards.

USP 71 Extending BUD

BUD: Microbiological Limits

- Most shelf life labels or listed expiration dates are used as guidelines based on normal handling of products.
- Use prior to the BUD does not necessarily guarantee the safety of the drug.
- Thus, immediately after the date, a CSP is not always dangerous nor ineffective.*
- Applied whenever an actual sterility test in accordance with USP Chapter <71> has not been performed

* Report 1 of the Council on Scientific Affairs (A-01) Full text: Pharmaceutical Expiration Dates. American Medical Association, June 2001. AMA Policy H-115.983

Understanding BUD

- Recognizes the probability of contamination even under best conditions:
  - Optimal employee performance
    - 0.1% (1 contaminated dose out of 1,000)
  - Contamination rates published in the literature
    - 0.3% – 16%
- Patient Safety: Protect patients from dangerous or even fatal overgrowths of microorganisms that may have been accidentally introduced
- Storage time: needs to be greater than zero but less than positive infinity*

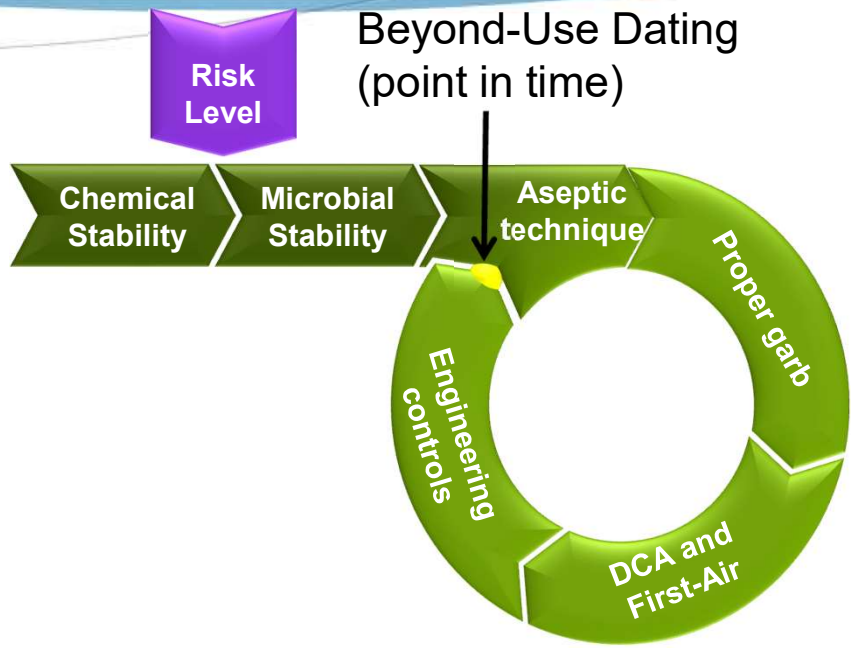
Understanding BUD

- For patient safety, BUD must be based on two factors:
  - Drug's chemical stability in conjunction with
  - Microbiological limits
- BUD will always be whichever is shorter
- Must factor in chemical stability
- Concern that microbial over-colonization of solutions would occur over time.
  - pH of solution is a consideration
    - Neutral (pH 6-8) favorable for microbial colonization

Microbiological Beyond-Use Dating

Risk Category	Room Temp	Refrigerator	Freezer (≤-10 °C)
Immediate Use	1 hour	1 hour	N/A
Low	48 hours	14 days	45 days
Low w/12-hr BUD	12 hours or less	12 hours or less	N/A
Medium	30 hours	9 days	45 days
High	24 hours	3 days	45 days

Understanding all of the elements



ASSUMPTION!
CSP is stored at its optimal temperature at all times.

Due to the inherent low probability that a Sterility Test can detect low levels of contamination in a batch, **sterility assurance must always be based** on process design and control.

Summary

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Flow of the Sterility Test

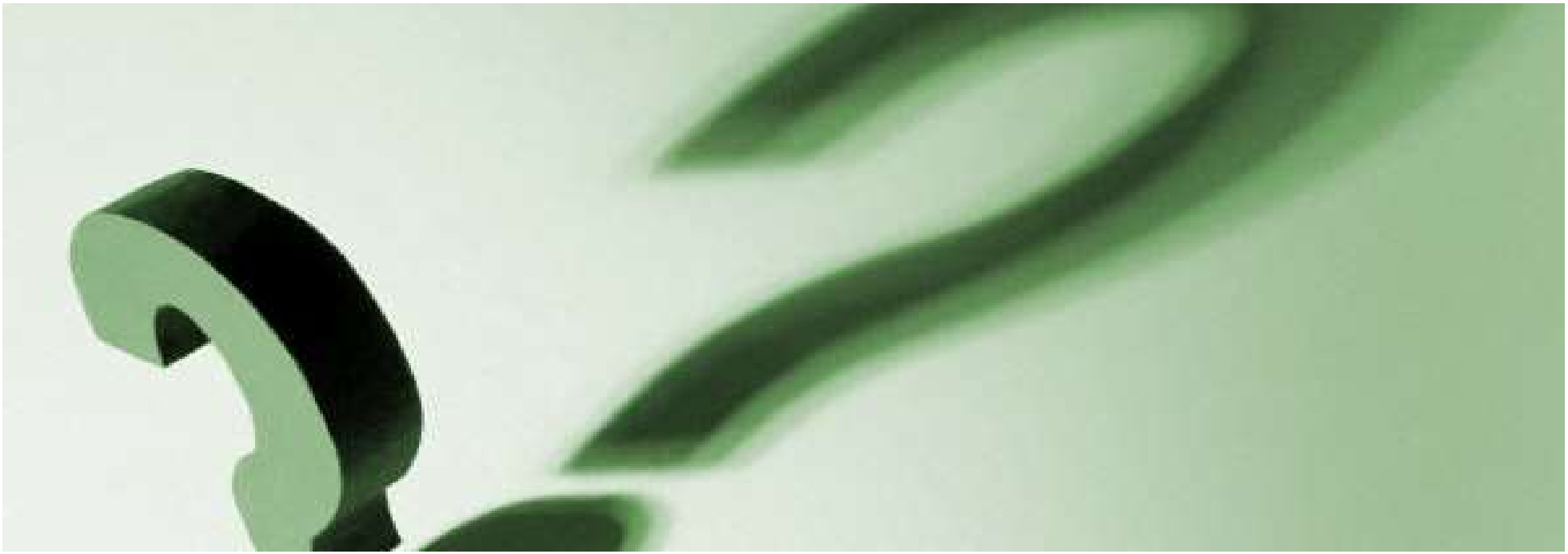
a) Media and Bacteriostasis/Fungistasis Testing (Method Suitability)
b) Eliminate any bacteriostatis/fungistatic properties
c) Determine number of articles, quantity from each, to test
d) Incubate the samples
e) Examine test articles for signs of growth
f) Examine suspect tubes microscopically for signs of growth
g) Subculture if necessary
h) Write the report

Reminder: All Compendial Microbiological Test Methods, including Sterility Tests, are Classical Growth Based Methods

Summary

- USP 71 Sterility Test
- Only Required when USP 797 Beyond Use Dating is exceeded
- No testing regardless of risk level required if USP 797 adhered too
- 2 Approved Methods
  - Membrane Filtration
  - Direct Inoculation
- Method Suitability Testing Required
- Growth Promotion Testing Required
- Process Simulation Testing not recognized by USP

Questions



Thank you

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