Changing Paradigms in Contamination Control

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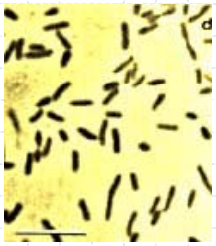
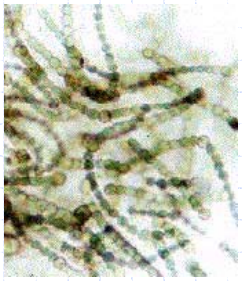
Presentation Objective

- This presentation will review current regulatory driven contamination control concerns in the healthcare industry highlighting the more controversial aspects.
- I will approach the subject from both the sides – regulatory & industry
- The views presented are solely my own and not those of the organizations responsible.

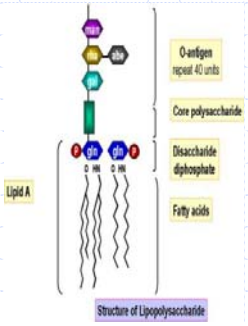
The Usual Suspects - Visually

Microorganisms



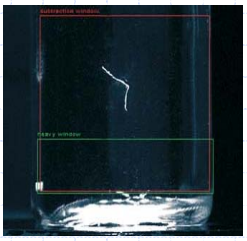


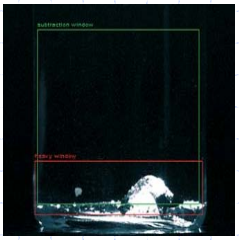
Pyrogens/Endotoxins

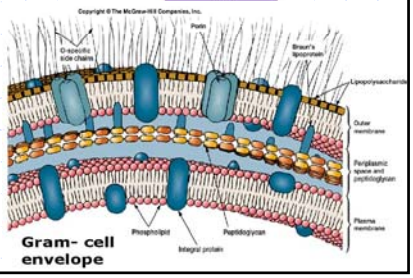


Structure of Lipopolysaccharide

Particles







The Usual Suspects

- Microorganisms & their associates
  - Bacteria, yeast
  - Mold
    - Transient
    - Resident
  - Viruses, prions, ??
- Pyrogenic Materials
  - Endotoxins
  - Exotoxins
- Particles
  - Glass fragments, delaminates, etc.
  - Fibers
  - ??

The Regulatory Landscape

- FDA – Part 211, guidances & **FD 483's**
- EMA – Annex 1 – Sterile Medicinal Products
- ISO – especially in the area of sterilization which is used by EMA to define requirements during inspections.
- ICH – as it relates to microbial control of non-sterile materials
- PDA – largely as an interpreter of others
- USP – charged to provide more basic science

FDA 21 CFR 211.113 a) & b)

(a) Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed.

(b) Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of all aseptic and sterilization processes.

Revised August 2008

Realities

- The concerns for microbial control have always been present. What has changed is the focus of attention now placed on it by regulators. FDA, EMA and others have all raised the level of concern.
- Other events have raised visibility as well:
  - 'Specified' microbes in non-sterile products.
  - Mold detection in all types of facilities.
  - Sterility concerns at generic manufacturers.
  - Visible particles in injectable drugs.
  - Viral contamination in biotech facilities.
  - Compounding pharmacy recalls.

Tension about **Perfection**

- There's a sense on the part of regulators that the practices used to control contamination have to absolute with respect to their outcome.
- That's simply not possible. We can never prove an absolute negative with respect to any of these contaminants.
- Our industry is extremely good at this and continually improving, but perfection is simply not attainable.

The Principal Players on the Field

PDA®
Parenteral Drug Association
Connecting People, Science and Regulation®

USP
U.S. Pharmacopeial Convention
Global Expertise
Trusted Standards
Improved Health

FDA

ISO

PDA®
Parenteral Drug Association
Connecting People, Science and Regulation®

PDA – TR 22 Revision – Media Fills

- Stresses the importance of procedural control for operator interventions for success.
- Introduces 'inherent' (process required) and 'corrective' (avoidable under the right circumstances) interventions to highlight the undesirability of tolerating excessive human interaction with sterilized equipment, components & products.
- Endeavors to clarify what 'participation' during a media fill means.
- Provides a detailed sequential description of media fill execution.

PDA – TR 61 - Steam In Place

- Defines practices for fixed equipment (bioreactors, product deliver systems, freeze dryers, etc.) to differentiate those from what is performed in autoclaves.
- The document makes distinctions between SIP processes that:
  - Sterilize – sterilization is the objective & validation requirements are comprehensive with BI's & TC's.
  - Sanitize – bioburden reduction to near zero is the objective & only temperature monitoring is performed.
  - Bioburden Reduction - bioburden reduction to defined limits is the objective & only temperature monitoring is performed.

PDA – TR 62 – Manual Aseptic

- Acknowledges the reality that a manual aseptic process is acceptable despite its inherent limitations.
- Outlines preferred practices for manual aseptic operations in cleanrooms & isolators.
- Stresses the importance of process design, optimization, rehearsal & training to refine the methods.
- Makes a distinction between LFH's (preferred for aseptic operations) & BSC's (acceptable only when worker protection is needed).

USP™
U.S. Pharmacopeial Convention

Global Expertise
Trusted Standards
Improved Health

USP <1211> Revision

- The new/revised chapter <1211> will cover only the topic of sterility assurance. The individual components of this include some existing content, and others identified only by title. Work will start here in 2014.
- An overarching new chapter <1229> devoted to general principles of sterilization of compendial articles has been developed and finalized.
- Eleven subchapters are planned – some of these are official now, others are mid-process and others are still to be written.

<1229.n> Series current status

Official in 1S to USP 36 – August 2013

<1229> ***Sterilization of Compendial Articles***

<1229.1> ***Steam Sterilization by Direct Contact***

<1229.2> ***Moist Heat Sterilization of Aqueous Liquids***

Official in 2S to USP 36 – December 2013

<1229.3> ***Bioburden Monitoring***

<1229.n> Series current status

Official in 1S to USP 37 – August 2014

<1229.4> *Sterilizing Filtration of Liquids*

<1229.7> *Gaseous Sterilization*

<1229.8> *Dry Heat Sterilization*

<1229.10> *Radiation Sterilization*

PF online Chapter Timeline

Chapter Title	Publication in *PF*
<1229.6> *Liquid Phase Sterilization*	*PF* 39(4) – July 1, 2013
<1229.11> *Vapor Phase Sterilization*	*PF* 40(1) – Jan 1, 2014
<1229.5> *Biological Indicators*	*Internal Draft In-process*
<1229.9> Physicochemical Indicators & Integrators	*Ready for PF*
<1229.X> *Sterilization In Place*	*In Planning*

Calculation of PNSU (SAL)

$$\log N_u = \frac{-F}{D} + \log N_0$$

where:

N_u = SAL / PNSU
D = D-value of the natural **bioburden**
F = F-value (lethality / dose) of the process
N_0 = **bioburden population**

The lethality is measured in units of time at the D-value condition. This calculation works for all processes where the D-value can be determined.

Calculation of PNSU (SAL)

$$\log N_u = \frac{-F}{D} + \log N_0$$

The bioindicator and physical measurements confirm this

The bioburden defines these

What is USP planning on BI's?

- Put appropriate BI content in one place, a subchapter of <1229>.
- Shift the thinking towards the use of the BI as a tool, rather than the process focus.
  - Require D-value & population be known for the purposes of control, but without the current arbitrary values.
- Provide clearer guidance on how they are to be used for validation & control of sterilization processes.

Where will USP's BI content be?

Monographs – Individual Monographs	<55> Biological Indicators—Resistance Performance Tests	< 1035> Biological Indicators For Sterilization	<1229> Sterilization Of Compendial Items
General Description	Total Viable Spore Count	Types of Bioindicators	
Packaging & Storage	D-value Determination Methods	Performance Evaluation	
Expiration Date		Use for In-process Validation	
Labeling			
Identification			
D-value			
Survival & Kill Window			
Total Viable Spore Count			
Purity			
Shipment			
Disposal			

<1228.n> Depyrogenation Series

- A separate series of chapters is planned on depyrogenation paralleling the <1229> sterilization model.
- Five different methods are anticipated:
  - Dry Heat Depyrogenation (separate from sterilization)
  - Separation (filtration)
  - Adsorption
  - Chemical Inactivation
  - Physical Removal (washing / rinsing)
- Recommendation is to eliminate the 3 log requirement and replacement with an empirical approach for all processes.

USP <61>, <62> & <1111>

- These chapters are to be used together, they are the result of PDG (Pharmacopeial Discussion Group, involving USP, EP and JP) activities.
- <61> Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests
- <62> Microbiological Examination of Non-sterile Products: Tests For Specified Microorganisms
- <1111> Microbiological Examination of Non-sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use

USP <61>

- <61> Microbiological Examination of Non-sterile Products: Microbial Enumeration Tests – bioburden test methods
- By itself this test raises no major concerns, because there are no defined / limits.

USP <62>

- <62> Microbiological Examination of Non-sterile Products: Tests For Specified Microorganisms – tests intended to detect the absence of specified organisms.
- This test also raises no direct concerns, because the limits are not stated within.

USP <1111>

- <1111> Microbiological Examination of Non-sterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use – recommended acceptance criteria for non-monographed products.
- This informational chapter defines what are **non-official** expectations for both <61> and <62>. Because it is numbered >1,000 it is informational & not a required compendial test! That distinction is generally ignored.

USP <1111> Table 1

Table 1. Acceptance Criteria for Microbiological Quality of Nonsterile Dosage Forms

Route of Administration	Total Aerobic Microbial Count (cfu/g or cfu/mL)	Total Combined Yeasts/Molds Count (cfu/g or cfu/mL)	Specified Microorganism(s)
Nonaqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	—
Oromucosal use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Gingival use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Cutaneous use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Nasal use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulization)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant Gram-negative bacteria (1 g or 1 mL)

USP <1111> Table 1 continued

- "Table 1 includes a list of specified microorganisms for which acceptance criteria are set. The list is not necessarily exhaustive, and for a given preparation it may be necessary to test for other microorganisms depending on the nature of the starting materials and the manufacturing process."
- The only certain way to ensure compliance would be to make non-sterile products in using 'sterile' technologies. **That's absurd.**

USP <1111> Table 1 continued

- "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: hazard varies according to the route of administration (eye, nose, respiratory tract).
  - The nature of the product: does the product support growth? does it have adequate antimicrobial preservation?
  - The method of application.
  - The intended recipient: risk may differ for neonates, infants, the debilitated.
  - Use of immunosuppressive agents, corticosteroids.
  - The presence of disease, wounds, organ damage."

USP <1111> Table 2

- Criteria are also provide for drug substances regardless of the means of production.

Table 2. Acceptance Criteria for Microbiological Quality of Nonsterile Substances for Pharmaceutical Use

	Total Aerobic Microbial Count (cfu/g or cfu/mL)	Total Combined Yeasts/Molds Count (cfu/g or cfu/mL)
Substances for pharmaceutical use	10^3	10^2

What's Wrong!!

- Comparable tests are not in place for all of the materials used to make these products.
- Even if there were, it wouldn't help much, because there are other factors contributing to the microbial content of both drug products & drug substances.
- Some help (perhaps not enough) is coming in the form of <1115> Bioburden Control of Non-sterile Drug Substances and Products. This chapter offers assistance on the important control practices.

What's Right, but Often Forgotten!

- Chapter <1111> is intended for non-sterile products and applying it to materials & API's used for sterile drug products is wrong.
- There are no references to 'specified microorganisms' in control of sterile drug products.
- The materials used for sterile drug products should be subject to bioburden and pyroburden controls.

Implications of Human Microbiome

- The 61, 62 & 1111 series of chapters were conceived before completion of the Human Microbiome Project.
- The results of the HMP raise important questions regarding the need to control microbial populations as aggressively as regulators have recently.
- A revisit of the entire issue seems to be necessary to make expectations more consistent with the real world data.

<1115> Bioburden Control

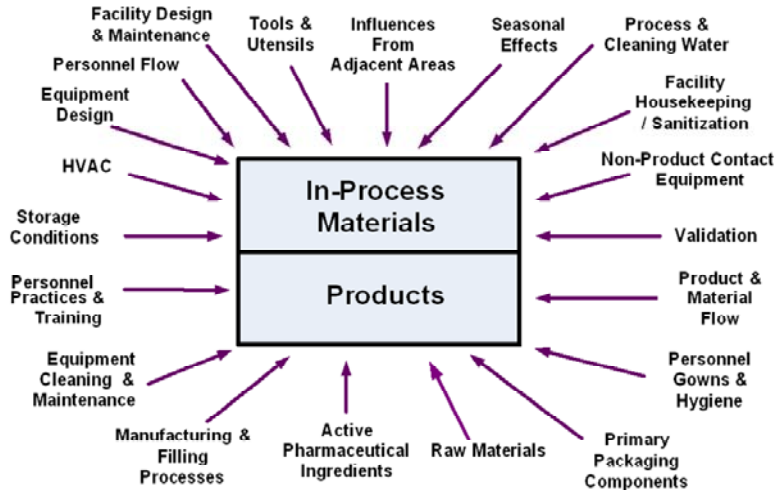
- Bioburden Control of Non-sterile Drug Substances and Products, *Pharmacopeial Forum* 39(4)
- In-process draft chapter that reviews the contributors to non-sterile product bioburden from a process as opposed to a monitoring perspective.
- In order of importance the control measure measures described are:
  1. Ingredient water
  2. Pharmaceutical ingredients
  3. Process equipment
  4. Manufacturing personnel
  5. Manufacturing environment.

<1115> Bioburden Control

- Environmental monitoring is de-emphasized in the draft chapter for several reasons:
  - PDA & other surveys indicated that there are many different facility design used in the manufacture of non-sterile drug substances and products.
  - As a consequence, the environmental programs varied widely as well.
  - Given the diversity of practice and uncertain implication of environmental contributions, USP believed that it should focus on microbial control as opposed to environmental monitoring.

<1115> Bioburden Control

Non-Sterile Product Microbial Influences



ISO

EN / ISO 14644-1: 2010

ISO classification number (*N*)	Maximum allowable concentrations (particles/m^3) for particles equal to and greater than the considered sizes shown below [a]					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
ISO Class 1	*10* [b]	d	d	d	d	e
ISO Class 2	100	*24* [b]	*10* [b]	d	d	e
ISO Class 3	1 000	237	102	*35* [b]	d	e
ISO Class 4	10 000	2 370	1 020	352	*83* [b]	e
ISO Class 5	100 000	23 700	10 200	3 520	832	e
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7	c	c	c	352 000	83 200	2 930
ISO Class 8	c	c	c	3 520 000	832 000	29 300
ISO Class 9	c	c	c	35 200 000	8 320 000	293 000

[a] All concentrations in the table are cumulative, e.g. for ISO Class 5, the 10 200 particles shown at 0,3 µm include all particles equal to and greater than this size.

[b] These concentrations will lead to large air sample volumes for classification. Sequential sampling procedure may be applied; see Annex D.

[c] Concentration limits are not applicable in this region of the table due to very high particle concentration.

[d] Sampling and statistical limitations for particles in low concentrations make classification inappropriate.

[e] Sample collection limitations for both particles in low concentrations and sizes greater than 1 µm make classification inappropriate, due to potential particle losses in the sampling system.

EN / ISO 14644-1: 2010

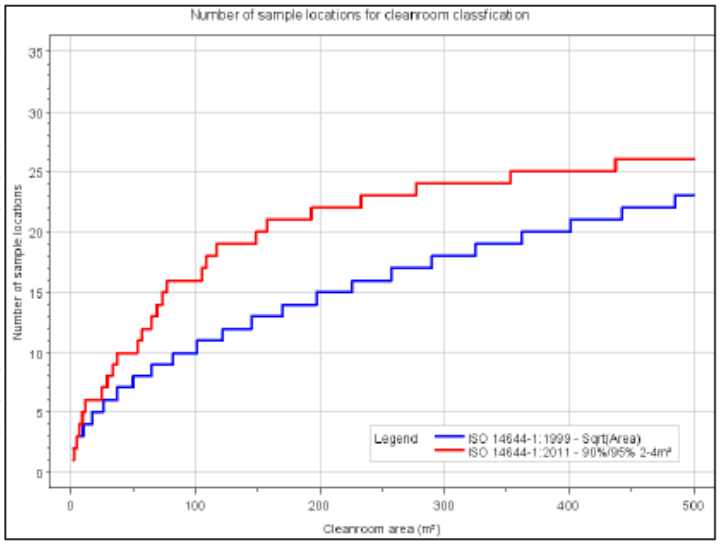


Figure 3. Comparison of the number of sample locations for cleanroom classification by ISO 14644-1:1999 and the new ISO/DIS 14644-1.

EN / ISO 14644-1: 2010

- For the statistical sampling principles in ISO 14644-1: 2010 to work correctly, sample locations should be selected randomly. Each point in the room must have the same probability of being selected.
- This is different from ISO 14644-1: 1999, where sample locations have been selected in a regular grid across the cleanroom.
- Classification under the new standard may not provide as much benefit as a precursor to initial environmental monitoring.

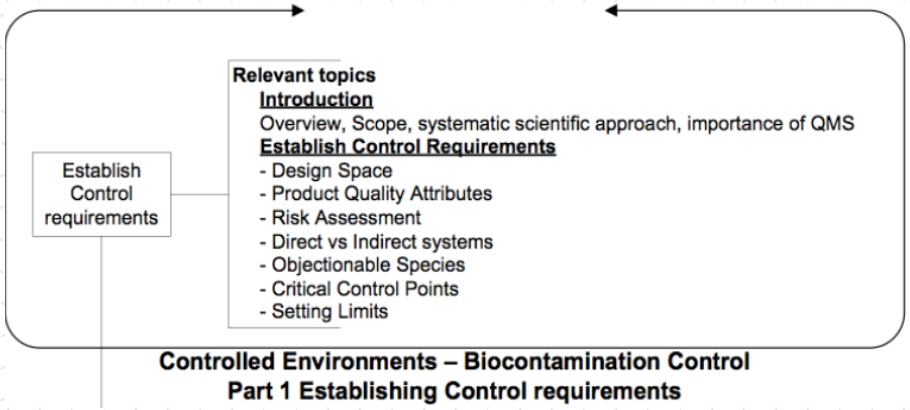
ISO 14698

- *Cleanrooms and associated controlled environments — Biocontamination control.*
- The ISO working group is tasked with creating a methodology to allow cleanrooms to be classified microbiologically. Premise is largely based on 'real-time' microbial detection.
- Many consider that the technical hurdles associated with this effort are not being adequately considered. A 'universal' approach seems ill conceived.

Objectives of ISO 14698

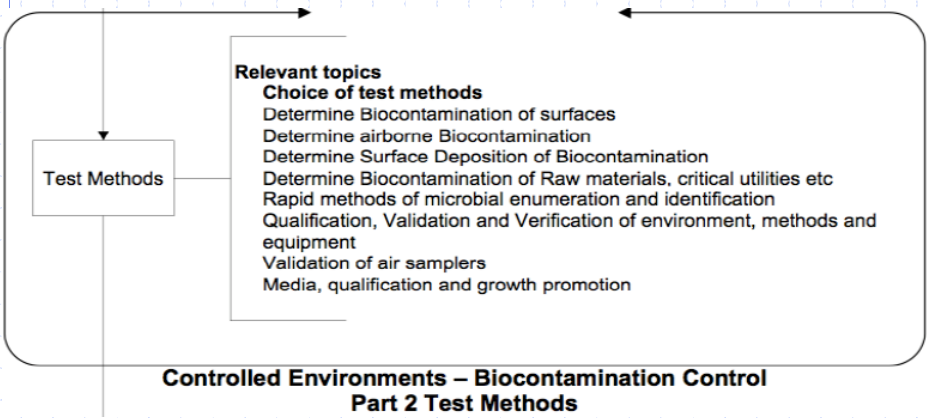
- Implement control requirements and refer to test methods and validation, particularly in relation to the quality and regulation of environmental factors and equipment.
- Provide a workable way of classifying cleanrooms for **ALL** applications.
- A 'third' standard on EM for classified environments. **Is it really needed?**
- Being pushed by UK, opposed by US.

ISO 14698-1 - Establish Control



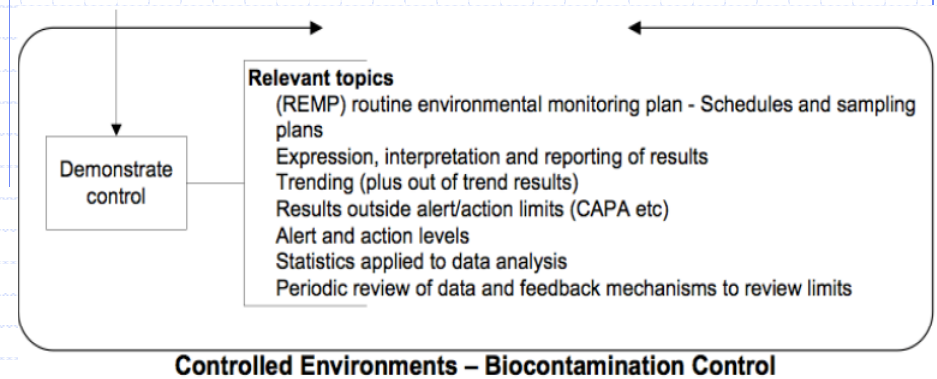
Controlled Environments – Biocontamination Control
Part 1 Establishing Control requirements

ISO 14698-2 – Test Methods



Controlled Environments – Biocontamination Control
Part 2 Test Methods

ISO 14698-3 – Demonstrate Control



Controlled Environments – Biocontamination Control
Part 3 Demonstrating Control

ISO 14698-3 – Air Limits?

Class	*Airborne limit In Operation cfu/m^3
ACV_x 1	<10#
ACV_x 2	< 100
ACV_x 3	< 1000
ACV_x 4	< 10000

1. *Combined with an appropriate incidence rate
2. 'x' is the species of interest
3. # still under discussion (added error by air collection method)
4. Intermediate classes are permitted, eg SVCx 3,5

ISO 14698-3 – Surface Limits?

Class	*Airborne limit In Operation cfu/m^3
ACV_x 1	<10#
ACV_x 2	< 100
ACV_x 3	< 1000
ACV_x 4	< 10000

1. *Combined with an appropriate incidence rate
2. 'x' is the species of interest
3. # still under discussion (added error by air collection method)
4. Intermediate classes are permitted, eg SVCx 3,5

EM Data is Meaningful

- FDA apparently believes that environmental monitoring is proof of contamination in the product.
- That's an unreasonable expectation on their part, yet industry has been is forced to pay increasing attention to results.
- It would be better to focus on microbial control through proper design and operating procedures.
- Monitoring is not a control mechanism, it's an alarm system!

Mold Detection is a Showstopper

- Detection of mold in the environment is considered a catastrophic event by some.
- Take a deep breath; it's not the end of the world.
- Must consider the relationship between the detection locale and production areas.
- It is essential to keep the facility clean and as dry as possible at all times.

Mold & its Implications

- There's distinctions in mold detection that matter.
  - Different molds in different locations is suggestive of external contamination tracked into the facility from various sources. While problematic, this is not a major issue.
  - Finding the same mold in multiple places, or different molds in the same place suggests a singular source. Investigation is needed to determine whether there is a resident population of mold(s) within the facility.

Particles in Injections

- There is an increasing expectation for the complete absence of particles of all types in injectables.
- No one disputes the desirability of that; however attaining complete absence may be impossible.
- Unlike microorganisms & endotoxin they can removed only by filtration. Particles on fill components can be formed after washing.
- The regulators are even expanding the concern to sub-visible particles.
- Just like microorganisms, controls aren't absolute.
- The patient safety implications of particles aren't definitive. Right now this is a bit of witch hunt.

Concluding Remarks

Keys to Success

- The important concerns in microbial control are universal for all products.
  - Man
  - Machine
  - Materials
  - Methods
- These have stated in 21 CFR CGMP requirements for decades.
- USP chapter <1115> describes practices for non-sterile products, but the basic principles are useable for sterile products as well.

Important Control Measures

- The use of cleanrooms is commonplace; don't loose sight of the key aspects of their operation
  - Microorganisms rely on carbon & H_20 to survive.
  - Keep things clean & dry as much as possible
  - Housekeeping is extremely important.
- Time limits are extremely important – especially when it comes to wet equipment and surfaces.

Don't Forget the Dark Side

- It's not enough to just consider the process areas. Look behind the scenes:
  - Mechanical spaces / machine rooms
  - HVAC systems
  - Interstitial space
  - Machine bases, etc/
- Keeping these locations reasonably clean and dry can help protect the nearby equipment and facility surfaces that are in closer proximity to the product.

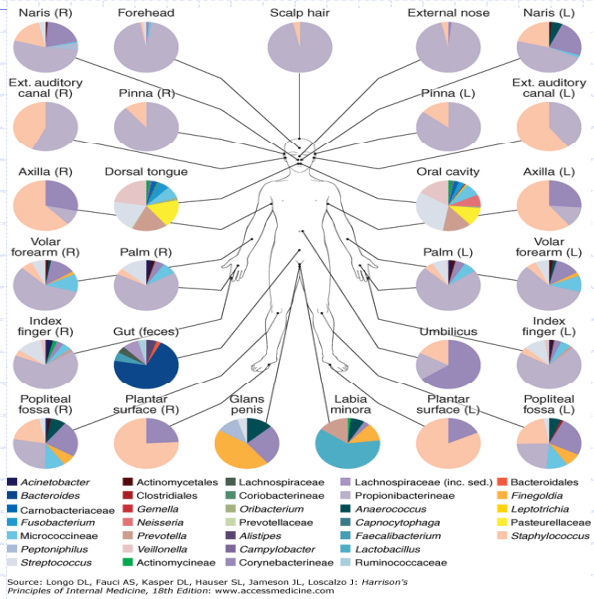
Modern Microbial Methods

- Rapid microbiology only gives the same uncertain results sooner than before.
- The limitations of sample size, intensity, frequency and recovery efficiency are unchanged.
- Fluorescence or real-time RNA/DNA tests may confirm the presence of microorganisms we should already understand are present. That knowledge doesn't change anything, though it might cause greater anxiety.

Human Microbiome Project

- It has been estimated that there are at least ten times as many bacteria as human cells in the body (approximately 10^{14} bacterial cells versus 10^{13} somatic cells).
- The majority of those bacteria are found in the gastrointestinal tract.
- Opportunistic pathogens were present in nearly every sample taken during the project. The levels of these were not high (0.1-1% of recovered organisms).

Human Microbiome by Genera



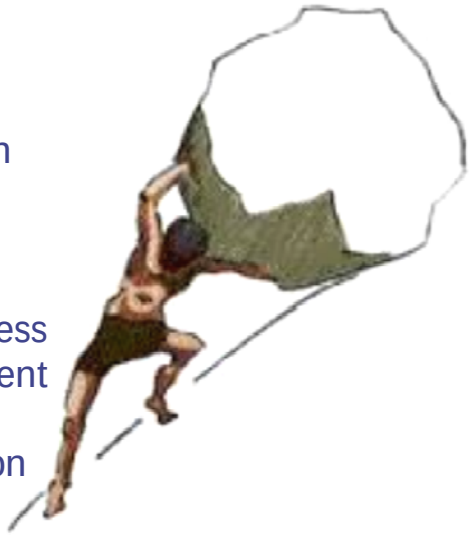
Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Abundant Opportunistic Pathogens

Location	Most Abundant PATRIC Pathogens (Descending Order)
Anterior Nares	*P. acnes; S. aureus; S. epidermidis; S. mitis ; Bacteriodes vulgatus ; Rothia mucilaginosa ; Gardinerella vaginalis ; Corynebacterium matruchoti*
Retroauricular Crease	*P. acnes; S. epidermidis; S. aureus; S. mitis ; B. vulgatus ; G. vaginalis; C. matruchoti*
Buccal Mucosa	*S. mitis; R. mucilaginosa ; P. acnes; C. matruchoti; Alistipes putredinis; B. vulgatus; G. vaginalis ; Bifidobacterium dentium; S. epidermidis*
Torgue Dorsum	*R. mucilaginosa ; S. mitis ; C. matruchoti; S. aureus; S. epidermidis; P. acnes; B. vulgatus; G. vaginalis; B. dentium*
Supra-gingival Plaque	*C. matruchoti; S. mitis; R. mucilaginosa*
Stool	*B. vulgatus; A. putredinis G. vaginalis; S. mitis, P. acnes; R. mucilaginosa: B. dentium; C. matruchoti; S. aureus; S. epidermidis*
Posterior Formix	*G. vaginalis; B. dentium; B. vulgatus; C. matruchoti; P. acnes; A. putredinis; S. mitis; R. mucilaginosa*

What Does This Mean?

- Efforts to eliminate microorganisms from products can never be 100% effective if personnel are involved in their production.
- It also means that extreme efforts to eliminate them are fruitless as they are already present in/on the patient!!
- Job security & frustration all in one.



Thanks for Your Attention!

Questions?

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