

INNOVATIVE TECHNOLOGIES ◦ SERVICE WITH RESULTS ◦ EXPERT TESTING



MANUFACTURING MICROBIOLOGY

Chad Ronholdt, B.Sc., MBA
Vice President Strategic Development

WWW.LABS-INC.ORG



AGENDA

- Origins of Microbiology
- Manufacturing Microbiology Fundamentals
 - Bioburden
 - Bacteriostasis & Fungistasis
 - Environmental Monitoring
- Advancements in Manufacturing Microbiology
 - Endotoxin
 - Sterility Testing
 - POU water testing

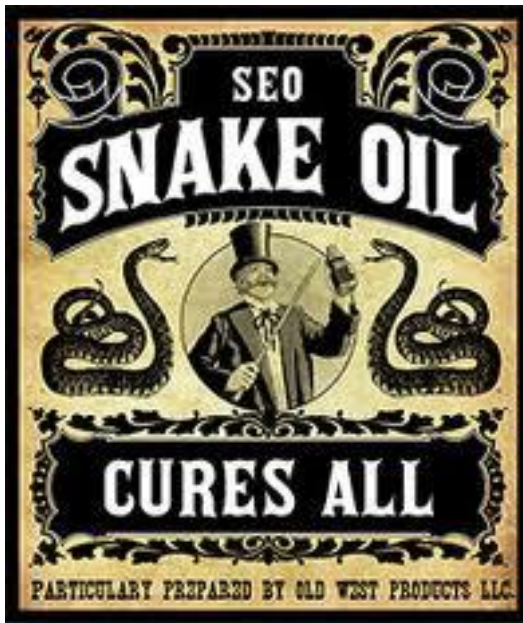
MICROBIOLOGY HISTORY

- Archaeobacteria emerged at least 3.5 billion years ago.
- Microorganisms were hypothesized to cause disease as far back as the Roman Empire 36 BC
 - “...*certain minute creatures which cannot be seen by the eyes, which float in the air and enter the body through the mouth and nose and there by cause serious diseases.*” Marcus Terentius Varro



EVOLUTION OF INDUSTRIAL MICROBIOLOGY

- The Food and Drug Administration (FDA) is the oldest comprehensive consumer protection agency in the U. S. federal government. Its origins can be traced back to 1848



- 1906 Pure Food and Drugs Act, a law that prohibited interstate commerce in adulterated and misbranded food and drugs
- Promulgated standards to ensure the safety and efficacy of various products that are described in United States Pharmacopeia (USP)



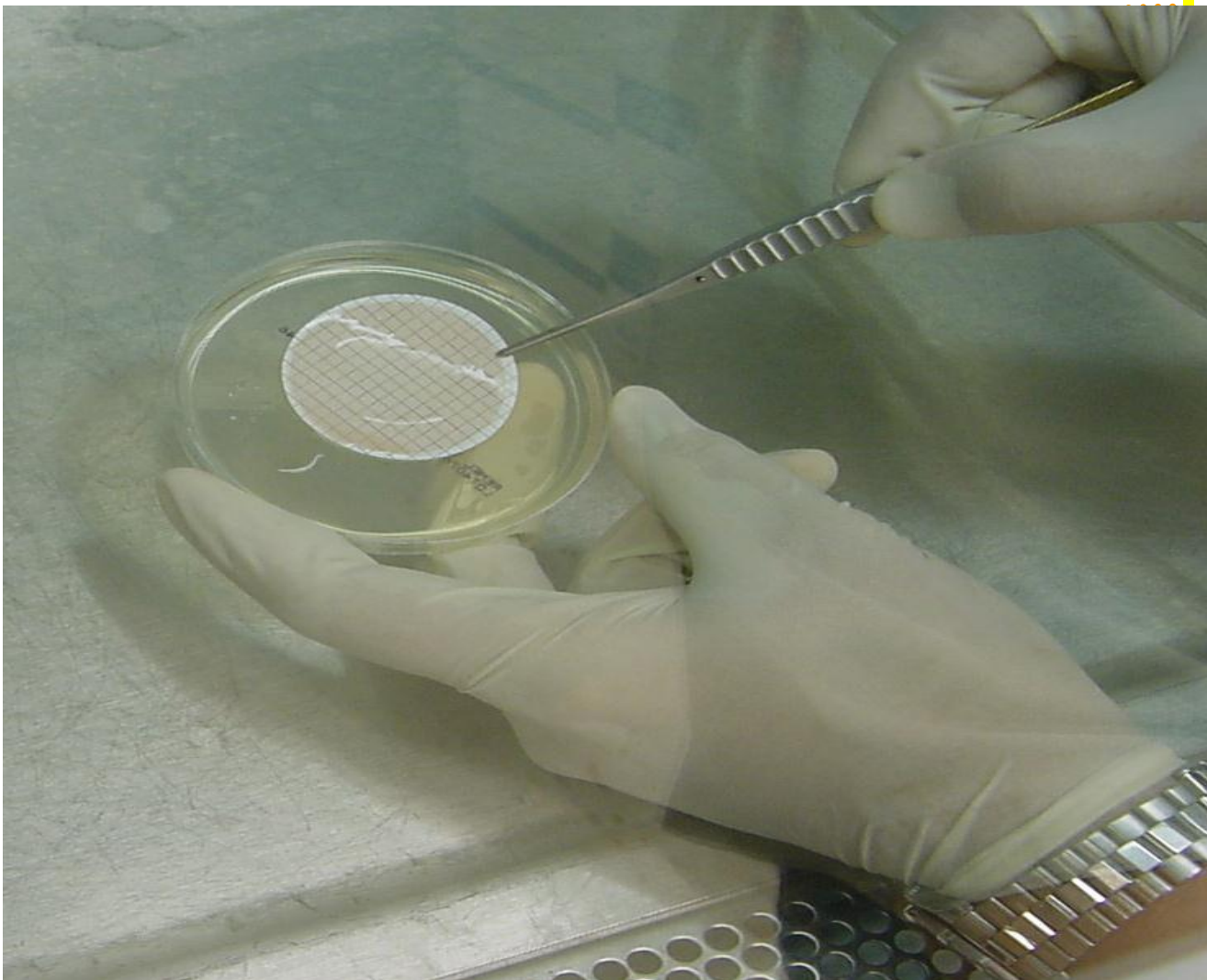
MICROBIAL RECOVERY (BIOBURDEN) USP <1227>; ISO 11737 PARTS 1&2

- Natural bioburden is defined as:
 - The amount of living microorganisms on an item (e.g. tissue or medical device) prior to and after manufacturing
 - Bioburden can be described both in terms of QUANTITY and TYPE of microorganisms
- Why is knowing your bioburden important?
 - Sterilization Dose
 - Aseptic processing
 - Process Capabilities
 - CONTROL!!!

SOURCES OF BIOBURDEN

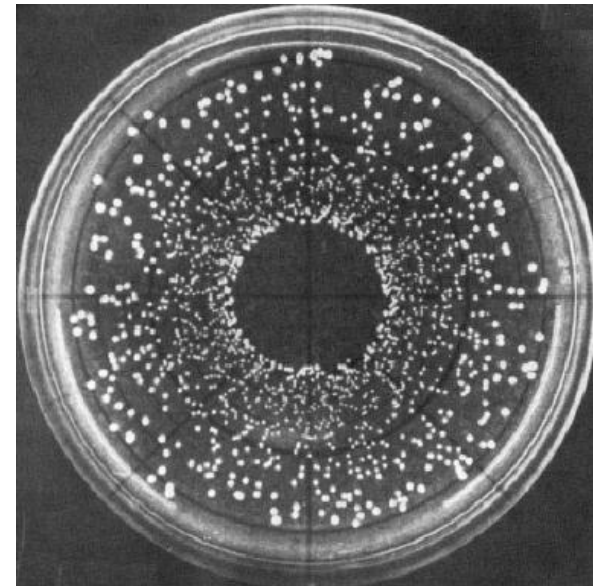


BIOBURDEN METHOD



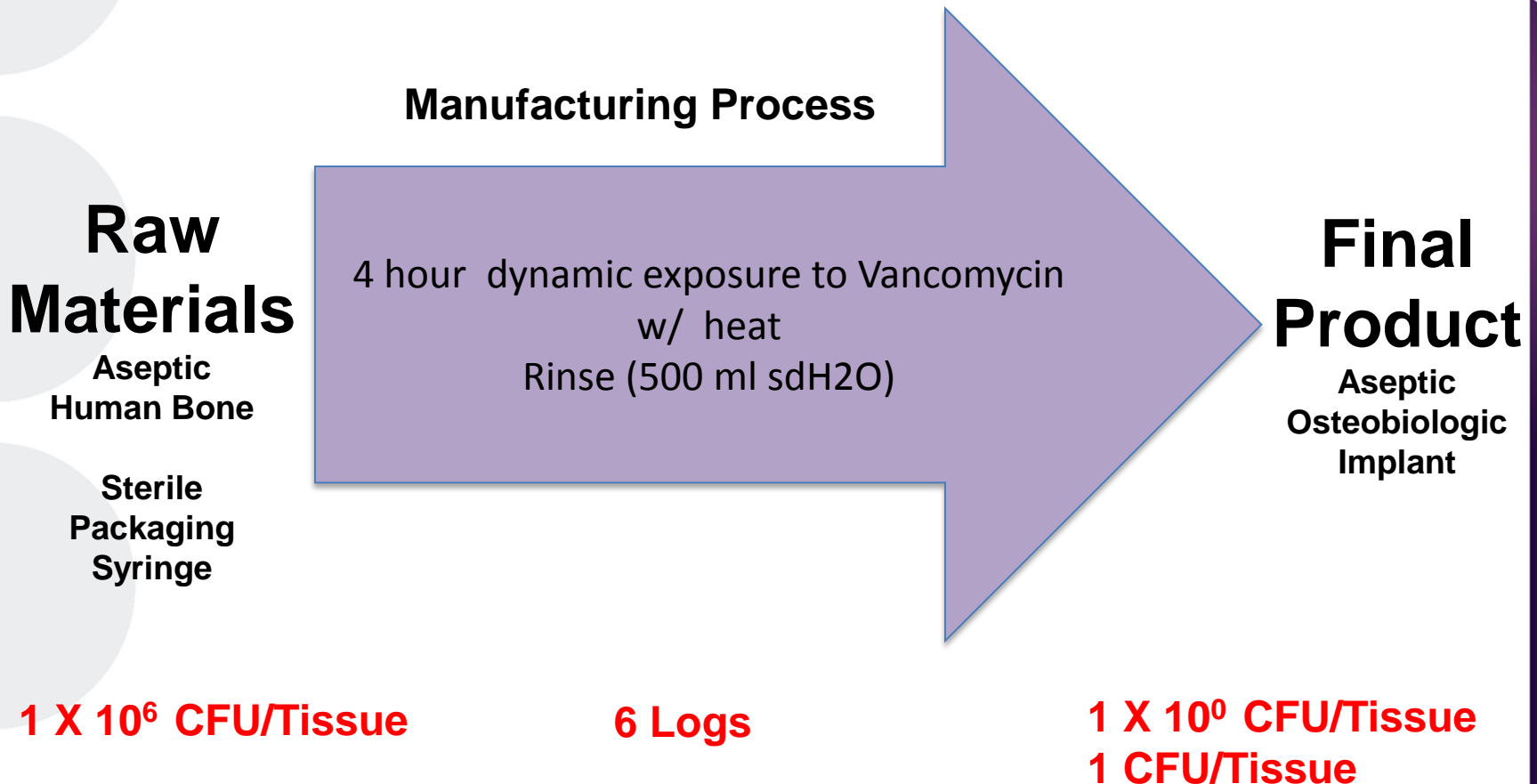
INCUBATION/IDENTIFICATION

- Aerobic plates (Tryptic Soy Agar)
 - 30 - 35°C for 7 days (initial read 24 – 72h)
- Fungal plates (Sabouraud Dextrose)
 - 20 - 25°C for 7 – 10 days (initial read 48 – 96h)
- Anaerobic plates (pre-reduced TSA w/ blood)
 - 30 - 35°C for 7 – 10 days (initial read 24 – 72h)
- Identification of colonies
 - Gram stain
 - Biochemical
 - Metabolic
 - Lipid analysis
 - 16s rRNA molecular



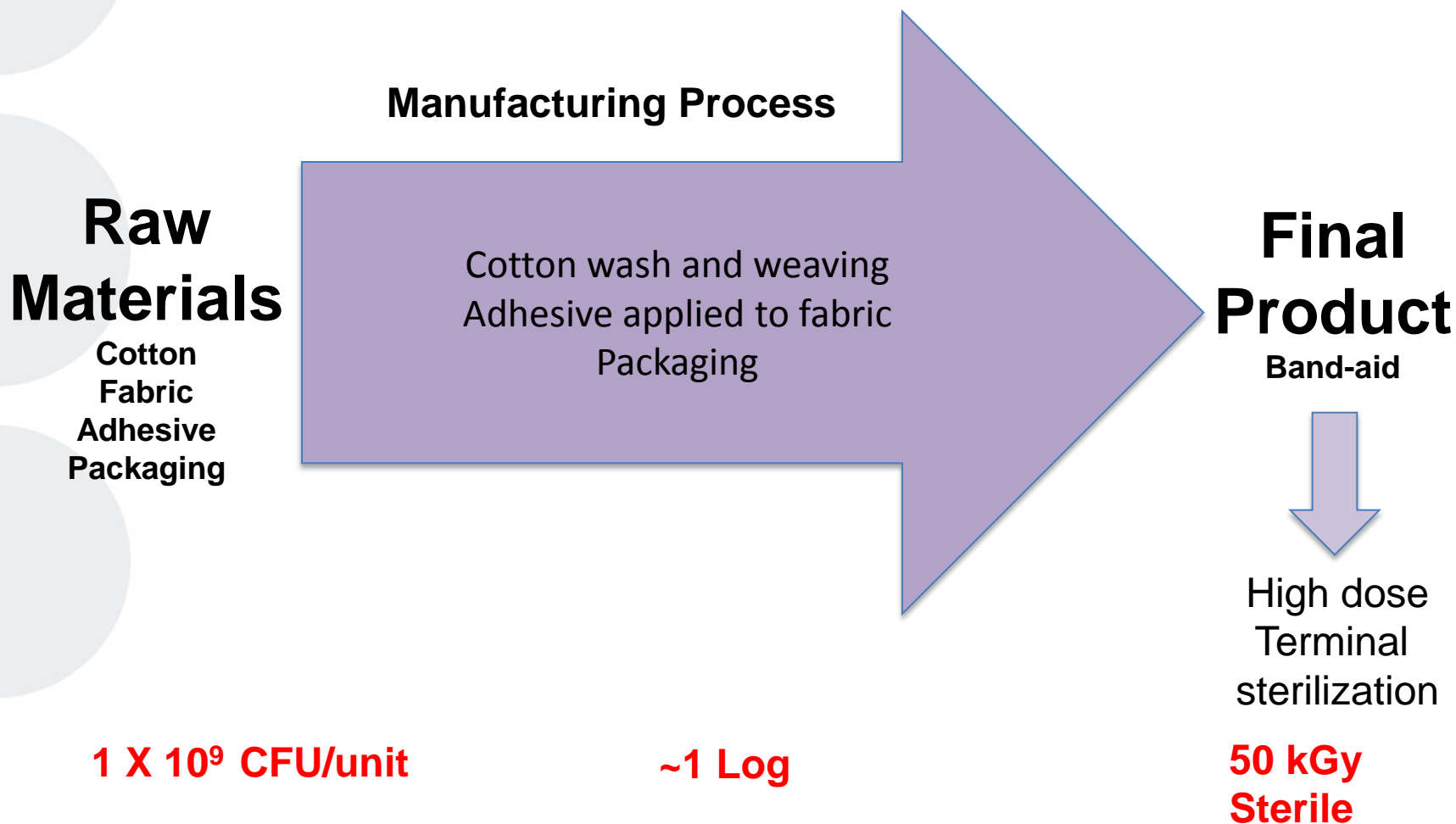


BIOLOGIC BIOBURDEN APPLICATION





DEVICE BIOBURDEN APPLICATION





BACTERIOSTASIS & FUNGISTASIS USP <71>

- Evaluates your product for inhibitory substances that will interfere or prevent the growth of any contaminant microorganisms during routine sterility testing
- 10 – 100 CFU
- Six Challenge Microorganisms
 - *Staphylococcus aureus* ATCC 6538
 - *Pseudomonas aeruginosa* ATCC 9027
 - *Bacillus subtilis* ATCC 6633
 - *Aspergillus brasiliensis* (formerly *A. niger*) ATCC 16404
 - *Candida albicans* ATCC 10231
 - *Clostridium sporogenes* ATCC 11437



ENVIRONMENTAL MONITORING

USP <1116>; ISO 14644 PARTS 1 - 7

- ISO guidance document specifies basic requirements for cleanroom operations. Evaluate the environment where the product is being manufactured
 - This includes room air quality, working surfaces, equipment and personnel
- For what?
 - Particles (viable and non-viable)
 - Surfaces
 - Static vs. Dynamic
- Cleanroom classifications (ISO 14644-1)
 - ISO 1 – 9 ($0 - 3.52 \times 10^7$, 0.5 μm particulates)

AIRBORNE EM TECHNIQUES

- To measure airborne contaminants the following items can be used:
 - Automated air sampler
 - Monitor velocity and volume of air exchanges
 - Monitor pressure differences (+ or -)
- Viable = microbial
- Non-viable = dust, oil, fibers, particulates



SURFACE EM TECHNIQUES

- To measure viable surface contaminants (e.g. bacteria and fungi) the following items can be used:
 - Swab
 - RODAC Contact Plates
 - Settle Plates





DESIGN OF SAMPLING PLAN

- Sampling Validation
 - Used to establish worst-case sample locations by level and type of bioburden present
 - (e.g. drains, gowning rooms, nooks/crannies)
 - Used to establish frequency of sampling
 - (e.g. daily, weekly, monthly, quarterly, annually)
 - Used to establish control and limits for critical areas
 - (e.g. powder fill room, device assembly room)





ALERT LEVELS

- Ranges that when exceeded indicate that a process may have drifted from its normal operating condition.
- Constitute a warning not necessarily a corrective action
- Establish control limit using statistical analysis of natural bioburden for each area and desired control level
 - 1 months of data (large standard deviation from mean)
 - 3 – 6 months of data (moderate standard deviation from mean)
 - 12 months of data (small standard deviation from mean)

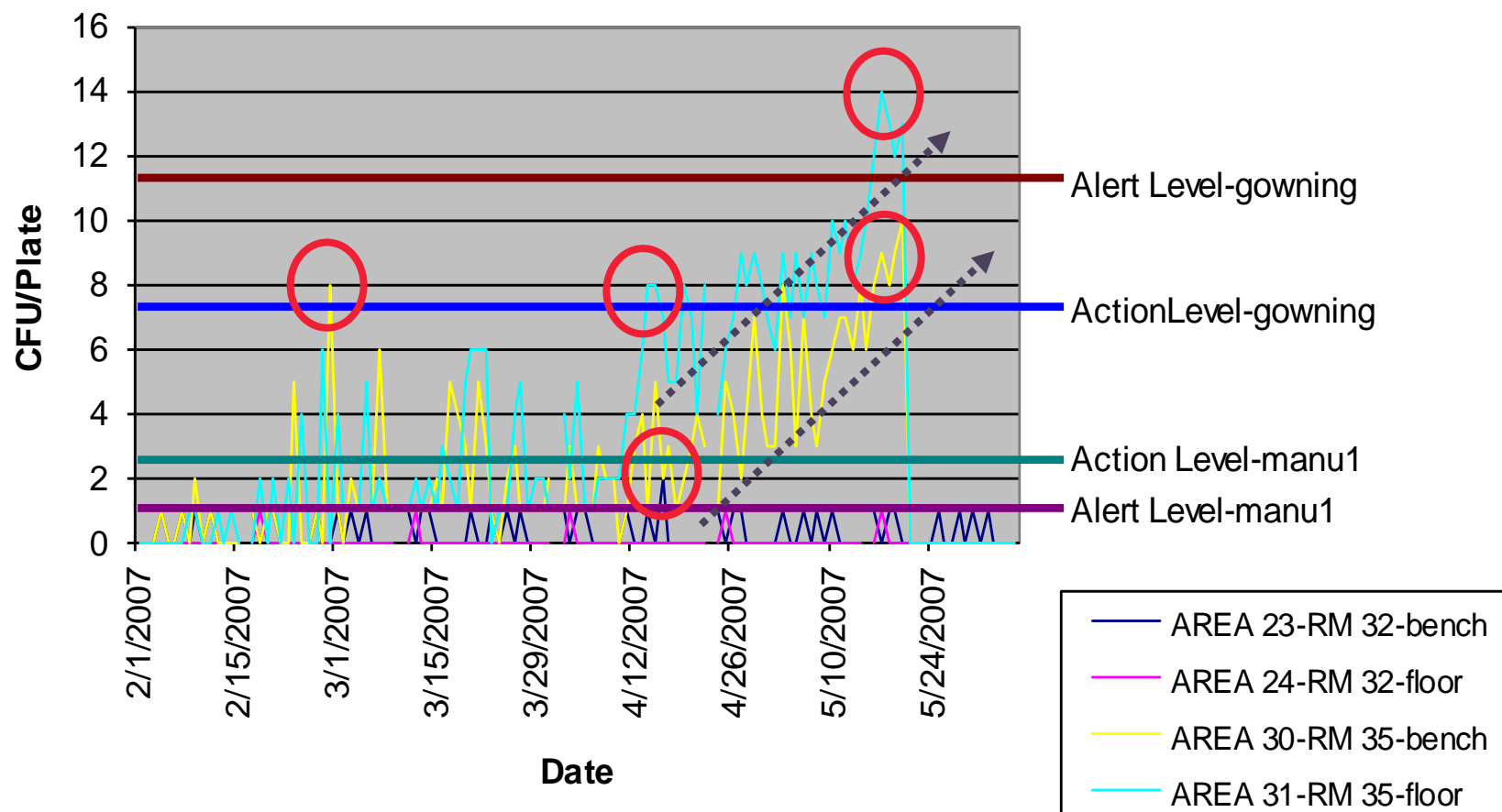


ACTION LEVELS

- Ranges that when exceeded indicate that a process may have drifted from its normal operating condition.
- Requires an investigation and corrective action to bring process back into normal operating range
- Establish control limit using statistical analysis of natural bioburden for each area and desired control level
 - 1 months of data (large standard deviation from mean)
 - 3 – 6 months of data (moderate standard deviation from mean)
 - 12 months of data (small standard deviation from mean)



RODAC EM 1 Feb - 5 June 2007





PERSONNEL MONITORING

- Gowning qualification
 - To verify that each technician can gown appropriately
 - Multiple RODAC plates (forearms, forehead, chest, fingers)
- Routine monitoring
 - To verify that each technician hasn't contaminated the product
 - Ensure continued gowning compliance
 - Trending and tracking of EM isolates
 - Multiple RODAC plates (forearms, fingers)





TAKE-HOMES

- Bioburden testing is one of the most versatile and important methods used today to establish control over numerous processes
- B&F is a critical element in the validation of your sterility test for lot release
- EM is one of the most frequently cited FDA 483 observations
- Use a combination of viable, non-viable and surface monitoring techniques as appropriate for your facility and product
- Let data drive your decisions

INNOVATIVE TECHNOLOGIES ◦ SERVICE WITH RESULTS ◦ EXPERT TESTING



ADVANCEMENTS IN MICROBIOLOGY

**ADAM WILSON
ASSOCIATE SCIENTIST
LABS INC.**

WWW.LABS-INC.ORG

AGENDA



- Endotoxin
 - FDA 1987 Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test For Human and Animal Parenteral Drugs, Biological Products, and Medical Devices
- Growth-Based Rapid Microbiological Methods
 - Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products (21CFR 610. 12)
- Point of Use Survey
 - PMF News letter. Volume 17, Number 8

INNOVATIVE TECHNOLOGIES ◦ SERVICE WITH RESULTS ◦ EXPERT TESTING



BACTERIAL ENDOTOXIN TEST (BET)



Background on Endotoxins

- Endotoxin
 - a heat-stable toxin associated with the outer membranes of certain gram-negative bacteria.
 - Endotoxins are not secreted but are released only when the cells are disrupted; they are **less potent and less specific than the Exotoxins.**
 - In large quantities they produce **hemorrhagic shock and severe diarrhea; smaller amounts cause fever, altered resistance to bacterial infection, leukopenia** followed by leukocytosis, and numerous other biologic effects.

History of Bacterial Endotoxin Testing

- **Prior to WWII**, Rabbit pyrogen test introduced
- **1956** Dr. Bang discovered that horseshoe crab blood forms clots when bacteria (*Vibrio* sp.) are present
- **1968** Dr. Bang and Dr. Levin created *Limulus* amoebocyte lysate, or LAL, and a new method to test for gram-negative bacteria
- **1971** LAL assay is developed
- **1977** FDA replaces Rabbit test with LAL test
- **1987** The FDA establishes guidelines for LAL testing of pharmaceuticals and medical devices.





ENDOTOXINS

- Endotoxins are of particular concern to those manufacturing pharmaceutical goods, biological products, and medical devices as they are one of the most potent pyrogens able to contaminate a product
- Most significant levels of endotoxin are predominantly found in water.
- Endotoxins are extremely resilient:
 - remain viable after steam sterilization, normal desiccation, and can pass through filters. **Research shows that temperatures in excess of 200°C for up to an hour** are required to remove endotoxin contamination.

SELECTION OF BET METHODOLOGY

- Gel Clot
 - Anything other than a firm gel is considered negative
- Chromogenic and Turbidimetric-End Point
 - the measurement of the turbidity or color after incubation at a fixed temperature and time period.
- Chromogenic and Turbidimetric-kinetic method
 - Measure the amount of time it takes for a series of standards to reach pre-determined optical density or color intensity



1987 FDA VALIDATION OF LAL TESTING



- This guideline sets forth acceptable conditions **for use of the Limulus Amebocyte Lysate test**. It also describes procedures for using this methodology as an end-product endotoxin test **for human injectable drugs (including biological products), animal injectable drugs, and medical devices**. The procedures may be used in lieu of the rabbit pyrogen test.
- **Drug Injection/Biological Products:**
 - At least three production batches of each finished product should be tested for inhibition and enhancement.
- **Medical Devices**
 - At least three production lots of each product
 - Recommends **2 devices for lot sizes under 30, 3 devices for lot sizes 30-100, and 3 percent of lots above size 100, up to a maximum of 10 devices per lot**.
 - If a device is to undergo extraction, a minimum extraction time should be **15 minutes at 37° C, one hour at room temperature (above 18° C)**
 - Each of the **10 test units should be rinsed with 40 mL**, of non-pyrogenic water.



WITHDRAWAL OF 1987 GUIDANCE.

“Today, FDA has the LAL Guidance for industry on validation of LAL test as an end product test for human parenteral drugs, biological products and medical devices. The 1987 Guideline is considered obsolete and does not reflect the Agency’s current thinking on the topic.

The United States Pharmacopeia (USP) publishes endotoxin testing recommendations and acceptance criteria in General Chapter <85> *Bacterial Endotoxin Test*. USP <85> provides methods and calculation of limits for drugs. FDA may, as needed, provide additional guidance to clarify the Agency’s current thinking on use of LAL, recombinant LAL, and other endotoxin testing methods.”

***Email from William McCornick, Division Director,
Division of product Quality OCBQ/CBER/FDA dated 12 July 2011.***



OTHER DOCUMENTS TO REFER TO:

- USP <85> Bacterial Endotoxins Test
- USP <161> Transfusion and Infusion Assemblies and Similar Medical Devices
- ANSI/AAMI ST72:2002/(R)2010 Bacterial endotoxins –Test methodologies, routine monitoring, and alternatives to batch testing



<85> BACTERIAL ENDOTOXINS TEST

- Chapter was harmonized with corresponding texts of European and Japanese pharmacopoeia.
- Explains preparation of solutions/testing
 - 4 lambda concentration standards for:
 - Inhibition/Enhancement testing
 - Routine Testing
 - **BET <85> does not recommend how many lots or samples should be tested for each product type**
- Determination of Maximum Valid Dilution
 - $$\text{MVD} = \frac{\text{Endotoxin Limit} \times \text{Concentration of Sample}}{\text{Lambda}}$$
 - Lambda=lowest point on the standard curve



<161> TRANSFUSION AND INFUSION ASSEMBLIES AND SIMILAR MEDICAL DEVICES

- Bacterial Endotoxins:
 - Proceed as directed under BET <85>
- Preparation of Devices
 - Select not less than 3 and not more than 10 devices
- Calculating Endotoxin limit
 - Formula= $(K \times N)/(V)$
 - K=Endotoxin allowed
 - N=Number of devices tested
 - V=Total volume of extract or rinse



ANSI/AAMI ST72:2002/(R)2010

- AAMI=Association for the Advancement of Medical Instrumentation
 - Written by AAMI Microbiological Methods Group
 - Members of the Endotoxin testing industry
- Compiled of multiple documents
 - FDA 1987 Guidelines for end-product testing of medical devices
 - Bacterial Endotoxin Testing <85>
 - <161> Transfusion and Infusion Assemblies & Similar Medical Devices
- AAMI
 - 2011 Standard is expected to be released in a few months for purchase

INNOVATIVE TECHNOLOGIES ◦ SERVICE WITH RESULTS ◦ EXPERT TESTING



VALIDATION OF GROWTH-BASED RAPID MICROBIOLOGICAL METHODS



HISTORY OF THE STERILITY TEST

- First published in the British Pharmacopoeia in 1932 for direct inoculation tests
 - in 1957 the use of **Membrane filtration** was added as an option for testing.
- Sterility test as a cultural medium growth test has remained relatively unchanged since 1960's
 - Weakness:
 - Small proportion of the batch is tested with the microorganism capable of growing under the test conditions
 - U.S, European, Japanese Pharmacopoeias were harmonized in 2009, the essential methodology remained unaltered.



RAPID MICROBIOLOGICAL METHODS (RMM)



- In February 2008: Guidance for Industry (FDA Draft guidance (non-binding recommendations))
 - What are Rapid Microbiological Methods (RMM)?
 - Why is Validation of RMMs necessary?
 - What products are included in this guidance?
 - How do I design the method comparison studies?



2008 GUIDANCE FOR INDUSTRY (FDA DRAFT GUIDANCE)

- **This guidance applies to somatic cellular therapy and gene therapy products. This guidance does not apply directly to human cells, tissues, and cellular and tissue products (HCT/Ps) which are described under 21 CFR 1271.10 or HCT/Ps which are regulated as medical devices under 21 CFR Part 820. HCT/Ps are not subject to the sterility testing provision in 21 CFR 610.12.**
- **However, HCT/P and device establishments seeking to validate an RMM may find these recommendations helpful.**

RAPID MICROBIOLOGICAL METHODS (RMM)



- June 21, 2011
 - Federal Register (Vol. 76, No. 119)
 - FDA proposed changes encourage the use of rapid methods for sterility testing of Biologics.
 - Description of the Proposed Rule:
 - The rule is intended to promote improvement and innovation in the development of sterility test methods by **allowing manufacturers flexibility needed for sterility testing of some novel products that may be introduced to the market.** Also to encourage the manufacturers to benefit from **scientific and technological advances** in sterility test methods as they become available.



2011 HIGHLIGHTS OF THE PROPOSED RULE

- **Elimination** of specified sterility test methods, culture media formulae, and culture media test requirements.
 - Sterility test be appropriate to the material being tested such that the material does not interfere with or hinder the test.
- **Elimination** of specified membrane filtration procedure requirements of certain products
 - oil products in water-insoluble ointments
- **Elimination** of bulk testing and focus on the final product
 - If final product is not available, test at some in-process stage, explain in BLA



2011 HIGHLIGHTS OF THE PROPOSED RULE

- Change the number of required test to examine of final container in filling.
 - Manufacturers to determine the appropriate sample volume and size for material being tested.
- Allow non-culture based methods
 - Molecular based methods: RT-PCR, Regular PCR, Adenosine Triphosphate (ATP) bioluminescence, chemiluminescence, and carbon dioxide head space measurement to detect potential viable contaminating microorganisms
- Eliminate specified incubation conditions (time/temp) and visual examination
 - Any validated sterility test method that is suitable to the material being tested.



PRINCIPLES OF THE VALIDATION

- “Validation of a microbiological method is the process by which it is experimentally established that the performance characteristics of the method meet the requirements for the intended application.”
- **Limit of Detection:** Reflects the lowest number of microorganisms that can be detected by the method in a sample matrix
- **Specificity:** Ability of the test method to detect a range of microorganisms necessary for the method to be suitable for its intended use.
- **Ruggedness:** The degree of reproducibility of results obtained by analysis of the same sample under a variety of normal test conditions, such as different analysts, different instruments, and different reagent lots.
- **Robustness:** The capacity of the test method to remain unaffected by small, but deliberate variations in method parameters.

2011 PRINCIPLES OF THE VALIDATION



- The new principles are a smaller scope of USP <1225> Validation of Compendial Methods

Typical Analytical Characteristics Used in Method Validation

1. Accuracy
2. Precision
3. Specificity
4. Limit of detection
5. Quantitation Limit
6. Linearity and Range
7. Robustness



VERIFICATION

Verification activities are necessary to demonstrate that sterility test methods can continue to reliably and consistently detect viable contaminating microorganisms.

- Culture-based test method
 - conduct tests to demonstrate that the performance of the test organisms and culture media are acceptable to detect the presence of viable contaminating microorganisms.
 - Growth Promotion testing
- Non-Culture-based test methods
 - Within the test itself, appropriate controls to demonstrate the ability of the test method to continue to reliably and consistently detect the presence of viable contaminating microorganisms.



Point of Use (POU) Testing of Pharmaceutical waters

- “PMF Water Testing Survey” conducted earlier this month.
- Have you replaced any POU sampling/testing with online sampling/testing or alternate microbiological methods?
 - 77% replied No
 - 23% replied yes
- What tests were replaced with online testing or alternate microbiological methods?
 - Conductivity
 - Total Organic Carbon (TOC)
- What are the biggest challenges to replacing traditional POU testing with alternate/online methods?
 1. Time/Resource required to implement online testing
 2. Management buy-in
 3. Determining ROI/Payback
- PHARMACEUTICAL MICROBIOLOGY FORUM NEWSLETTER- VOL. 17 (8)

TAKE HOME MESSAGE FOR UTILIZING ENDOTOXIN TESTING AND USING RMM



- BET
 - For medical device testing, labs can refer to general chapters <85>, <161> and AAMI
 - New AAMI standard to be released in the next couple months
 - Drugs and Biological testing: Use general chapter <85>

- RMM
 - If you are going to alternate from the USP method <71>, the compendial method should be your benchmark.

 - The proposed changes are to make the testing better fit the materials (samples) being tested.

 - If a lab decides to utilize alternate methods than what is established in their BLA, the lab must submit a BLA supplement.



REFERENCE

- United States Pharmacopeia 34 and National Formulary 29 (USP 34 NF29), Transfusion and Infusion Assemblies and Similar Medical Devices, General Chapter 161, pp. 117 (2011)
- United States Pharmacopeia 34 and National Formulary 29 (USP 34 NF29), Validation of Compedial Methods, General Chapter 1225, pp. 779 (2011)
- ANSI/AAMI ST72:2002/®2010 Bacterial endotoxins-Test methodologies, routine monitoring, and alternates to batch testing pp.17 (B.3.1)
- U.S. Food and Drug Administration. Guidance for Industry: Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products. (February 2008)
- Federal Register/Vol. 76, No.119/Tuesday, June 21, 2011:www.gpo.gov/fdsys/pkg/FR-2011-06-21/.../2011-15346.pdf
- Westney, R. Result of the “PMF Water Testing Survey”. Pharmaceutical Microbiology Forum Newsletter, 17 (8)
- Sandle, T. Sterility Test Requirements for Biological products. Pharmaceutical Microbiology Forum Newsletter, 17 (8)

INNOVATIVE TECHNOLOGIES ◦ SERVICE WITH RESULTS ◦ EXPERT TESTING



**THANK YOU!
QUESTIONS?**

cronholdt@labsinc.org; 720.528.4784

adam_wilson@labs-inc.org; 720-528-4729

Join our monthly newsletter: www.labs-inc.org/newsletter/

WWW.LABS-INC.ORG