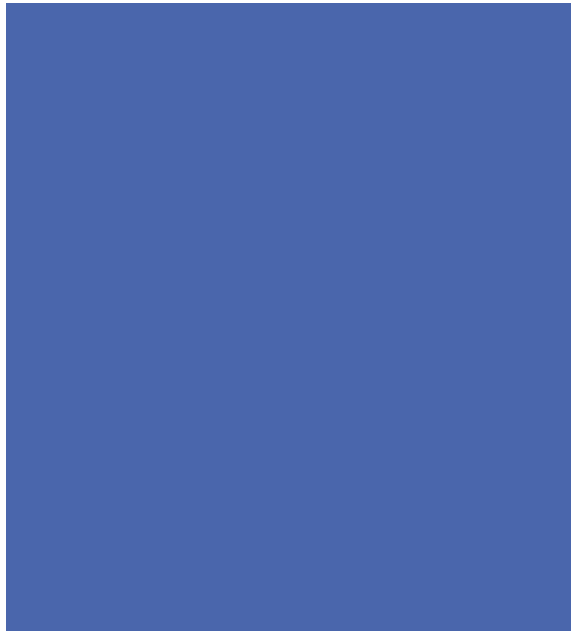
Topic 1 :
Environment monitoring



Guidance and Regulations

Part I – Topic 1

Course Objectives

1. Provide understanding of contamination control, EM and risk management and how to utilize risk assessments specifically for EM program

2. Provide instruction for developing EM programs for different product types based on risk and regulatory expectation

What is Environmental Monitoring?

- Sampling of controlled environments for non-viable and viable air particulates as well as surface viables
- Allows for assessment of effectiveness of cleaning/disinfection
- Allows for identification of trends
- Facilitate early detection of potential problems

What is Contamination Control?

- A proven system that prevents external contaminations from entering the controlled environments

Cleaning and Sanitization?

- Controls contamination that is introduced to the cleanrooms

Regulations and Guidance

- ISO 14644-1 "Cleanrooms and Associated Controlled Environments"
- USP 1116 "Microbiological control and Monitoring of Aseptic Processing Environments "
- USP 1115 "Bioburden Control of Nonsterile Drug Substances and Products "
- FDA Aseptic Processing Guideline
- EU Annex 1
- AAMI TIR 52:2014 –Environmental Monitoring for the Manufacture of Terminally Sterilized Healthcare Product
- PDA TR 13 "Fundamentals of an Environmental Monitoring Program"

Regulations and Guidance cont'd

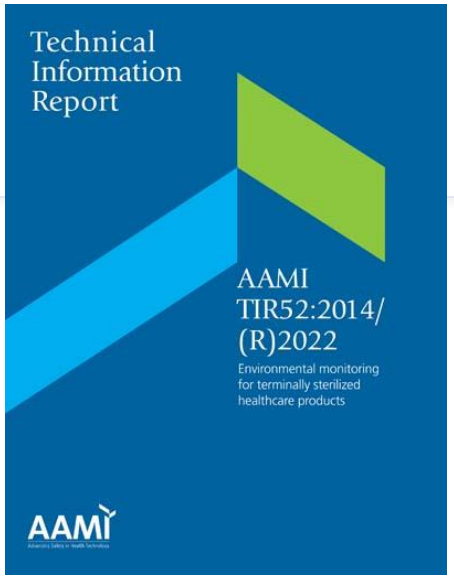
- **Aseptic Processing :**
  1. FDA Aseptic Processing Guidance Document – Sept 2004
  2. EU Annex 1,
  3. USP 1116

Regulations and Guidance cont'd

- **Low Bioburden, Cell Therapy/Gene Therapy:**
  1. EU Annex 2 : Manufacture of biological active substances and medicinal products for human use
  2. USP 1115 Bioburden control of nonsterile drug substance and products
  3. EU Guidelines on GMP specific to Advanced therapy Medicinal Products (ATMP)
- **Non-Sterile**
  1. EU Annex 2
  2. USP 1115

Regulations and Guidance cont'd

Medical Device:



- AAMI TIR on Environmental Monitoring
  - Published in April 2014
  - Revision in 2022
- TIR 52 Environmental Monitoring for the Manufacture of Terminally Sterilized Healthcare Products

USP <1116>

- This Chapter is specific to EM in the aseptic core only
- Risk based sample locations and frequencies with documented rationale
  - Table 2 suggests sampling frequency in relation to product risk
- Rate of occurrence of excursions in place of CFU levels
  - Contamination recovery rates – percentage of plates showing any microbial recovery regardless of the number of CFU
  - Alert and action levels are defined relative to these percentage

USP <1115>

- Describes Microbial Assessment of Non-sterile Product Manufacturing Environments
  - Part of Risk-Based Microbiological Control Program
- Contamination likely depends on level of human activity and levels of gowning
- Sampling locations should be selected based on risk evaluation
- Frequency of monitoring should reflect the potential risk associated with the dosage form
  - Products that are resistance to microbial contamination require little to no monitoring

Regulations and Guidance Viable Air Monitoring

- FDA Aseptic Processing Guidance
- EU Annex 1

Critical Area – Class 100 (ISO 5)

- …critical because an exposed product is vulnerable to contamination and will not be subsequently sterilized in its immediate container …

Critical Area – Class 100 (ISO 5)

- Air in immediate proximity of exposed sterilized containers/closures and filling/closing operations would be of appropriate particle quality … 0.5 micron particles NMT 3520 particles per cubic meter …= ISO 5 …. when counted at representative locations, within the airflow, and during the filling/closing operations

Critical Area - Class 100 (ISO 5)

- ….We recommend that measurements to confirm air cleanliness in critical areas be taken at sites where there is the greatest potential risk to the exposed sterilized product, containers and closures.

FDA Sterile Drug Products Produced by Aseptic Processing Guidance
cont'd

Environmental Monitoring

- … it is especially important to monitor the microbiological quality of the critical area to determine whether or not aseptic condictiones are maintained during filling and closing activities.
- Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production.
- Critical surfaces that come in contact with the sterile product should remain sterile throughout an operations.
- Critical surface sample should occur at the conclusion of aseptic processing

Monitoring Methods

- Surface – touch plates, swabs, contact plates

Monitoring Methods

- **Active Air** – impaction. centrifugal, membrane, etc.
- …the air sampler should be evaluated for its suitability for use in an aseptic environment … ability to be sterilized, and disruption of unidirectional airflow

Monitoring Methods

- **Passive Air** – Settle Plates
- Because only microorganisms that settle onto the agar surface are detected, settle plates can be used as qualitative or semi-quantitative
- Their value in critical areas is enhanced by ensuring that they are positioning in locations posing the greatest risk of product contamination
- The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples

FDA Sterile Drug Products Produced by Aseptic Processing Guidance

cont'd

- Table 1 – Air Classifications

Classifications	ISO	> 0.5 micron particles /m^3	Microbial Active Air Action Levels CFU / m^3	Microbial Settle Plate Action Levels CFU/4 hr (90 mm)
100	5	3,520	1	1
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

EU Annex 1 Highlights

- Clean rooms and clean air devices should be classified in accordance with ISO 14644-1.
- Classification should be clearly differentiated from operational process EM.
- Table with maximum permitted airborne particle concertation for each grade – **AT REST, IN OPERATION** for both 0.5 micron and 5.0 micron particle sizes for each Grade A, B, C, D… (D in operation levels not defined)…

EU Annex 1 Highlights cont'd

Table 1 : Maximum permitted total particle concentration for classification

Grade	Maximum limits for total particle ≥ 0.5 um/m³		Maximum limits for total particle ≥ 5 um/m³	
	At rest	In operation	At rest	In operation
A	3,520	3,520	Not specified[a]	Not specified[a]
B	3,520	352,000	Not specified[a]	2,930
C	352,000	3,520,000	2,930	29,300
D	3,520,000	Not Predetermined [b]	29,300	Not Predetermined[b]

- [a] Classification including 5 um particles may be considered where indicated by the CCS or historical trends
- [b] For Grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable .

EU Annex 1 Highlights cont'd

Table 2 : Maximum permitted microbial contamination level during qualification

Grade	Air sample CFU / m^3	Settle plates (diameter 90 mm) CFU/4 hours (a)	Contact plates (diameter 55 mm) CFU/plate
A	No growth		
B	10	5	5
C	100	50	25
D	200	100	50

- (a) Settle pates should be exposed for the duration of operations and changed as required after a maximum of 4 hours. Exposure time should be based on recovery studies and should not allow desiccation of the media use.

EU Annex 1 Highlights

Table 5 : Maximum permitted total particle concentration for monitoring

Grade	Maximum limits for total particle ≥ 0.5 um/m³		Maximum limits for total particle ≥ 5 um/m³	
	At rest	In operation	At rest	In operation
A	3,520	3,520	29	29
B	3,520	352,000	29	2,930
C	352,000	3,520,000	2,930	29,300
D	3,520,000	Not predetermined [a]	29,300	Not predetermined [a]

- [a] For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and on routine data, where applicable.

EU Annex 1 Highlights

- Where aseptic operations are performed, monitoring should be frequent using methods such as settle plates, volumetric air and surface sampling (e.g., swabs and contact plates).
- Sampling methods used in operation should not interfere with zone protection.
- Results from monitoring should be considered when reviewing batch documentation for finished product release.
- Surfaces and personnel should be monitored after critical operations.
- Additional microbiological monitoring is also required outside production operations, e.g., after validation of systems, cleaning and sanitization.

EU Annex 1 Highlights

- Great emphasis on use of Quality Risk Management and Risk Assessment throughout the document
- Contamination control strategy as part of the lifecycle with ongoing and periodic review and update
- Air visualization studies should be considered when establishing the facility EM program

Clean room and clean air device qualification

- Clean rooms should be qualified according to the characteristics of the environment.
- Each manufacturing operation requires an appropriate environmental cleanliness level in the operational state in order to minimize the risks of particulate or microbial contamination of the product or materials being handled.
- Classification is a method of assessing the level of air cleanliness against a specification. Classification per ISO 14644 series.
- Classification is a part of the qualification of a clean room.

EU Annex 1 Highlights

- For initial classification the minimum number of sampling locations can be found in ISO 14644-1.

- However, a higher number of samples and sample volume is typically required for the aseptic processing room and the immediate adjacent environment (Grade A/B) to include consideration of all critical processing locations such as point of fill stopper bowls.

EU Annex 1 Highlights

- Clean rooms should be qualified periodically and after changes to equipment, facility or processes based on the principles of QRM.
- For Grade A and B zones, the maximum time interval for requalification is 6 months.
- For Grades C and D ….12 months
- HEPA Filter Testing

EU Annex 1 Highlights

Viable and non-viable environment and process monitoring

- Environment and process monitoring is part of the overall contamination control strategy…

EM

- For Grade A monitoring,… sample at locations of the highest risk of contamination to the sterile equipment surface, container-closure and product….

EU Annex 1 Highlights

Non-viable monitoring

- The particle limits given in the table for the "at rest" state should be achieved after a short clean up period defined during qualification in an unmanned state after completion of operations

- *So …you need to know you can achieve those 5 micron levels that were dropped for classification*

- Although monitoring of ≥5.0 µm particles are not required for room qualification and classification purposes, it is required for routine monitoring purposes as they are an important diagnostic tool for early detection of machine, equipment and HVAC failure.

Viable monitoring

- Where aseptic operations are performed, microbiological monitoring should be frequent using a combination of methods such as settle plates, volumetric air, glove print and surface sampling (e.g., swabs and contact plates)

- Sampling methods should not pose a risk of contamination to the manufacturing operations.


Time for
a Break!!

Classification of Cleanroom and EMPQ

Part I – Topic 2

Presentation Overview

- Background ISO 14644 Revision
- Changes to ISO 14644 Part 1
- Changes to ISO 14644 Part 2
- Impact
- Risk Assessment Requirements

ISO 14644 Background

- The first document of ISO 14644 was published in 1999, ISO 14644-1
- In 2000, ISO 14644-2 was published, which began the process of FED-STD-209E being cancelled.
- The US General Services Administration (GSA) released a Notice of Cancellation for FED-STD-209E, Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones, on November 29, 2001 and FED-STD-209E was then superseded by ISO 14644-1 and ISO 14644-2

ISO 14644 Background cont'd

- In December 2012, revisions of ISO 14644-1 and -2 were released as Draft International Standards.
- In September 2014, a second edition of revisions to ISO 14644-1 and -2 were released as Draft International Standards.
- August 27, 2015 FDIS released for final 2 month vote
- Passed – new version released and published 12/15/15

ISO 14644 -1 : 2015 Highlights

- Title change
- Exclusion of particles ≥ 5 microns from the classification table for ISO class 5
- Determination of sample points required for classification of a cleanroom
- Locating sample points within a cleanroom
- Removal of 95% locations
- Dealing with super huge cleanrooms
- Instrument Calibration

Title Change

- ISO 14644-1 1999 Classification of air cleanliness
- ISO 14644-1 2015 Classification of air cleanliness by particle concentration

Removal of ≥5 micron particle at ISO 5

- Removing the ≥ 5 micron particle concentration in ISO 5
- Note – In the old FS 209E, Class 100 did not have a 5 micron testing requirement
- Belief was that there is uncertainty associated with particle collection efficiency and accuracy of counting low concentrations. Potential particle loss in the sampling system
- 14644-1:2015 provides a mechanism of extrapolating the macro-particle descriptor for class limits of 20 and 29 particles ≥ 5 µm
- Impact to Annex 1 requirements

Removal of ≥5 micron particle at ISO 5 , 1999 version

ISO 14644-1:1999 Table 1 – Selected airborne particulate cleanliness classes for cleanrooms and clean zones

ISO classification number (*N*)	Maximum concentration limits (particles/m³ of air) for particles equal to and larger than the considered sizes shown below (concentration limits are calculated in accordance with equation (1) in 3.2)					
	0,1 µm	0,2 µm	0,3 µm	0,5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1 000	237	102	35	8	
ISO Class 4	10 000	2 370	1 020	352	83	
ISO Class 5	100 000	23 700	10 200	3 520	832	29
ISO Class 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO Class 7				352 000	83 200	2 930
ISO Class 8				3 520 000	832 000	29 300
ISO Class 9				35 200 000	8 320 000	293 000

NOTE Uncertainties related to the measurement process require that concentration data with no more than three significant figures be used in determining the classification level

Removal of ≥5 micron particle at ISO 5 , 2015 version

ISO 14644-1:2015 Table 1 – ISO Classes of air cleanliness by particle concentration

ISO Class number (*N*)	Maximum allowable concentrations (particles/m³) 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
1	*10*[b]	d	d	d	d	e
2	100	*24*[b]	*10*[b]	d	d	e
3	1 000	237	102	*35*[b]	d	e
4	10 000	2 370	1 020	352	*83*[b]	e
5	100 000	23 700	10 200	3 520	832	d, e, f
6	1 000 000	237 000	102 000	35 200	8 320	293
7	c	c	c	352 000	83 200	2 930
8	c	c	c	3 520 000	832 000	29 300
9g	c	c	c	35 200 000	8 320 000	293 000

Determination of Sample Points

- Use a lookup table – replacing the equation (square root area)
- Number of sample locations increases
- The new approach allows each location to be treated independently with at least a 95% level of confidence that at least 90% of the cleanroom or clean zone areas will comply with the maximum particle concentration limit for the target class of air cleanliness

Remove 95% Upper Confidence Limit

- The requirements to calculate the 95% upper confidence limit(s) for 2 to 9 sample locations was removed

Positioning of Sample Locations

- Find the minimum number of sample locations from Table A.1
- Then divide the whole cleanroom or clean zone into sectors of equal area
- Then select within each sector a sample location **representative of the characteristics of that sector**
- At each location, position the particle counter probe in the plane of the work activity
- Additional sample locations may be selected for locations considered critical

Impact of Requiring Representative Samples

Cleanroom or clean zone layout

Equipment

Airflow systems

HEPA filter locations

Return Vent Locations

Directly under a HEPA may not be representative

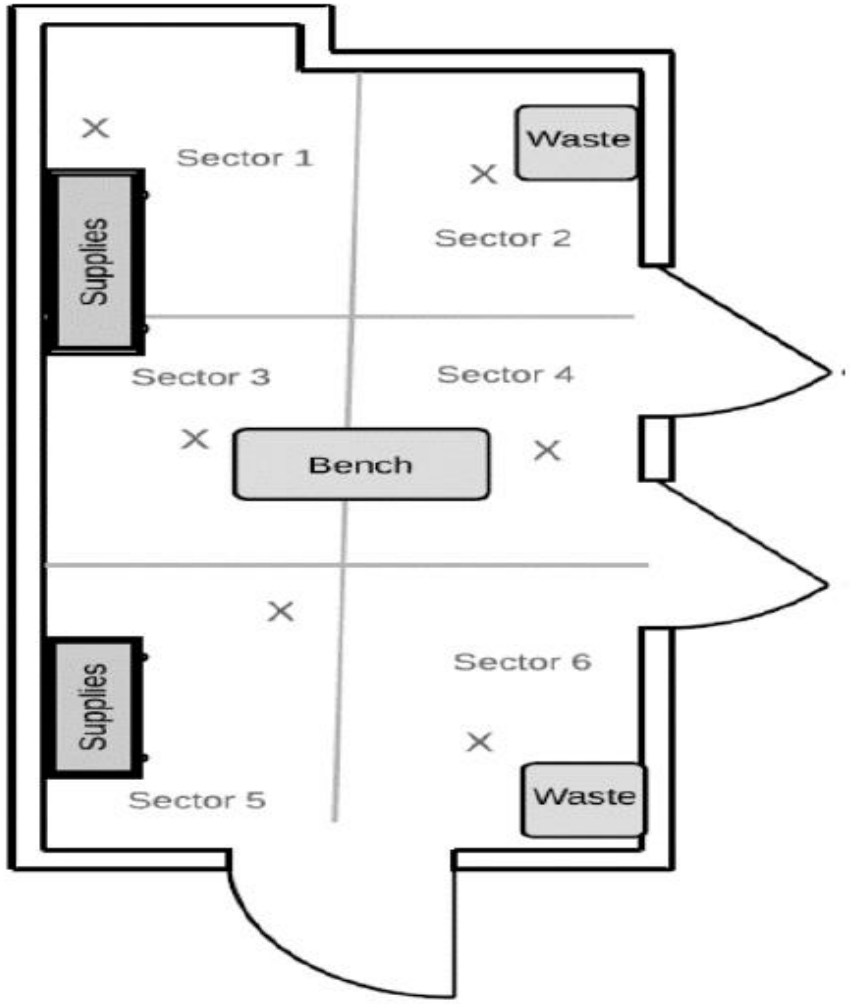
How Do You Handle?

- Map your rooms
- Justify locations – How?
  - Decision Tree
  - Add additional locations based on risk for critical activities
- Impact to contractors?
- What happens when you move things in the room?
- Feed into your EM Risk Assessment

Case Study ISO 14644-1

Room Number	Room Description	Square Feet	Square Meters	Square Meters, rounded up	Required sectors
1201	Gowning Room	137	12.7277	13	6

Case Study ： Map Gowning Room



Case Study ： Room Assessment Example

Room Number	Room Description	Sector	HEPA?	Return Vent?	Equipment?	Materials?	Personnel traffic?
1201	Gowning	1	NO	YES	NO	YES	YES
		2	NO	NO	NO	YES	YES
		3	YES	NO	NO	YES	YES
		4	YES	NO	NO	YES	YES
		5	NO	NO	NO	YES	YES
		6	NO	NO	NO	YES	YES

Case Study : Sample Locations Example

Room Number	Room Description	Sector	Sample Location
1201	Gowning	1	Next to the light in front of the return
		2	In front of the garbage can
		3	Under the light and sprinkler
		4	Under the sprinkler between men's and women's locker rooms
		5	In front of shelving on dirty side of bench
		6	In front of men's room locker door on clean side bench

Larger Cleanrooms

- Use the *Table A.1 – Sampling locations related to clean room area*, for cleanroom up to 1000 m^2
- Use equation for larger cleanrooms

Particle Counter Calibration

- The requirement that all light scattering airborne particle counters be calibrated to ISO 21501-4 : 2007 criteria
  - Guidance on what to do if you cannot meet this
  - Document rationale for using instrument

ISO 14644-2 Changes

- Emphasizes the need to consider a monitoring strategy in addition to the execution of the classification of a cleanroom or clean zone
- As you collect more data after initial classification, your on-going monitoring will help you better assess how your cleanroom operates
- Principal – gain assurance your cleanroom performs as expected after classification.

ISO 14644-2 : 2015 Highlights

- Title Change
- Monitoring Plan
- Risk Assessment
- Periodic Classification
- Alarms

Title Change

- ISO 14644-2 : 1999 Specifications for testing and monitoring to prove continued compliance with 14644-1

- ISO 14644-2 : 2015 Monitoring to provide evidence of cleanroom performance related to air cleanliness by particle concentration.

Risk Assessment

- ISO 14644-2 specifies the requirements of a monitoring plan, **based on a risk assessment of the intended use**.

- A risk assessment shall be undertaken to
  - Develop a monitoring plan by determining what factors may your ability to maintain your classification air cleanliness levels
  - Determine the monitoring requirements to provide evidence of performance

Monitoring Plan

- Guidance given for :
  - Creation of the Plan
  - Use of Risk Assessment
  - Review and approval of your monitoring plan
  - Implementing
  - Data analysis
  - Review the monitoring plan periodically
  - The plan should reflect the level of air cleanliness required, critical locations and performance attributes of the cleanroom

Monitoring Plan cont'd

- List and justify parameters to be monitored
  - Including those that may affect the airborne particle concentration
- Describe and justify measuring methods
- Identify and justify sample locations
- Establish alarms and/or alert/action levels
  - Explain what will be done if out of limits data found
- Establish the need and frequency of periodic cleanroom classification
- The format for recording data
- Trending methods
- Reporting requirements
- Frequency of review of the monitoring plan

Periodic Classification

- Periodic classification shall be undertaken annually

- The frequency can be extended based on risk assessment, the extent of the monitoring system, and data that are consistently in compliance with acceptance limits or levels defined in the monitoring plan

Annexes

- Annex A – Matters to consider when developing a monitoring plan
  - Select a risk assessment tool
  - Pressure differential monitoring
  - Airborne particle monitoring system
  - Airflow velocity and volume monitoring
- Annex B – Setting Alert and Action Level
- *Both are informative annexes*

Impact of ISO 14644-2 Changes

- Perform a risk assessment based on your HVAC and cleanroom performance
- Create a monitoring plan based on results of the risk assessment – what might contaminate my cleanroom and how/when will I monitor this?
- Determine and justify your periodic classification testing frequency based on the risk assessment results
- Determine and justify other testing (recovery, leak test, etc.)
- HUGE opportunity to leverage your day to day data to support your testing frequencies.

Monitoring Plan Development – Risk Assessment

Annex A

- Select an appropriate tool
- HACCP, FMEA, PHA, FTA, HAZOP, etc.

Monitoring Plan Development – Risk Assessment cont'd

Annex A

- Define required performance and operating conditions that may need to be monitored
  - Factors such as
    - Understand contamination sources and their impact on the activity in the cleanroom
    - Performance of HVAC that may affect cleanliness levels – pressure differentials, airflow uniformity, airflow volume, ventilation effectiveness, temperature, RH

Monitoring Plan Development – Risk Assessment cont'd

Annex A

- Normal and energy-saving set-back mode
- At rest or operational states
- Occupancy and level of activity – including change of shift

Monitoring Plan Development – General Consideration

Annex A

- Measuring system being used
  - Accuracy, calibration
- Measuring technique
  - Manual or automated
- Location of monitoring system components
  - Access for PM and Calibration
- Instrument/sample probe location, configuration and orientation
- Frequency of sampling to detect excursions

Monitoring Plan Development – General Consideration cont'd

Annex A

- Factors that can impact the monitoring system
  - Cleaning procedures/agents, fumigation, temperature, humidity, product or material hazards
- Any potential adverse impact of the sampling system on the process or environment
  - Pulling too much volume from an air sampler in a small space
- Smoke study results
- Ventilation effectiveness in the rooms
  - Air change rates, room recovery, clean-up times

Monitoring Plan Development – General Consideration cont'd

Annex A

- Impact of extent/frequency of cleaning on particle levels
  - During cleaning, immediately after cleaning
- Process activities that may impact the environment (setup)
  - Recovery time after activity?
- Personnel positions and movements during production
- Number and role of personnel in the cleanrooms
- Impact of equipment generated particles
  - Conveyor belt abrasion, sealing glass ampules, welding of tubing

Monitoring Plan Development – General Consideration cont'd

Annex A

- Data Management
  - Includes data integrity, storage and retrieval
- Establishing techniques to assess and evaluate data
  - Trending, creating trend reports
- Development of alert and action levels
- Requirements for commissioning and testing the monitoring system(s)
- Requirements for PM of the monitoring system(s)

Monitoring Plan Development – General Consideration cont'd

Annex A

- Pressure Differential Monitoring
  - Managing fluctuations caused by door openings or use of local exhaust
  - Establishing alert and action levels that are sensitive to normal pressure fluctuations (door openings/closings)
  - Manual or automated monitoring of pressure diffs?

Monitoring Plan Development – General Consideration cont'd

Annex A

- Airborne Particle Monitoring System
- Determining the system configuration needed for real-time systems
  - *Do I need multiple point of use units or single system with manifold and transport tubing (could impact 5 micron particles)*
  - Collection efficiency
  - Suitability to monitor selected sizes
  - Accessibility for PM, calibration , repair
  - Manual or automated monitoring of pressure diffs?
  - Air sample flow rates and volumes
  - Frequency and duration of sample collection
  - Sample probe orientation

Monitoring Plan Development – General Consideration cont'd

Annex A

- Airflow Velocity and Volume Monitoring
- Determining the airflow velocity or volume measurement technique
- Determining the location of the measurement device so it is representative of the system being monitored
  - You may have to evaluate multiple locations to prove measurements are representative


TIME FOR A
BREAK

EM
Performance Qualification

Part I – Topic 3

1st Step

- Perform EM Risk Assessment
- Use Classification Work as a starting point
- The locations for baseline and EMPQ should be selected based on risk

Initial Baseline EM Study

- Post Construction : Clean
- Pre Initial Cleanroom : Disinfectant Cleaning
- Collect samples at all or some of the EM Risk Assessment Selected Locations
  - Gain understanding on what you have as a baseline that you are trying to kill with your initial cleanings
  - Serves as In-Situ Data for Disinfectant Efficacy Studies

Continued Baseline Post Cleaning

- Continue to collect baseline EM samples
- Perform Triple Cleaning of Facility and collect samples in between each cleaning to show knock down of microbial levels
- Perform mock operations in cleanrooms to gain confidence in your ability to pass EM PQ
  - Is my cleaning working?
  - Are my operators behaving as to be expected?
  - Am I certain I can obtain the levels I expect under static and dynamic conditions?

EM PQ

- Collecting EM Samples during Static and Dynamic conditions
- Number of runs?
  - Some suggest 3 Static and 3 Dynamic
  - Base on Risk
  - Base on Baseline Data
- Be ready to start EM PQ
  - Common Mistakes
    - Rush for business pressure and then fail
    - Oversample out of fear

Routine EM Program Start Up

- Close out successful EM PQ reports
- Roll into same sample locations
  - Add clause to EM PQ that will continue to collect as EM Start UP
- Make EM program SOPs official
- Suggest same number of sample locations used in EM PQ for 3, 6 or 12 months
  - Gain knowledge from data
  - Cut back for routine EM once confident in results

Disinfectant Efficacy

- Use ATCC to initiate
- In-house isolates are a must but suggest wait 1 year for seasonal variation to perform with these
  - How do you know what they are until your collect over time?

Environmental Control

- Pharmaceutical Manufacturing generally involves complex and multi-step systems
  - Utilities
  - Manufacturing Systems
  - Environment
  - Personnel

Each bringing significant risks for microbial contamination


TIME FOR A BREAK

Cleaning and Disinfection

Part I – Topic 4

Microbial Contamination Control Strategies

1. Remove or destroy contamination in product
2. Prevent microorganisms from contaminating the product
3. Combination of 1 and 2

Cleaning and Disinfection

- Two activities which are not the same thing – often confused as the same thing

Cleaning and Disinfection

- Two activities which are not the same thing – often confused as the same thing

Cleaning and Decontamination

- **Cleaning** : Physical removal of dirt and foreign materials – including microorganisms, particulates and chemicals or residuals that can build up from disinfectants

- **Cleaning process** : A process that is used to remove any product, process related material and environmental contaminant introduced into equipment as part of the manufacturing stream. (PDA TR 29)

- **Cleaning purpose** : Periodically done to reduce particulates residue, bioburden and **prepares surfaces for disinfection** and **deactivation**.

Decontamination

- **Decontamination** : removal or inactivation of microbiological or chemical contamination

- **Decontaminant** : the process of cleansing an object or substance to remove contaminants such as micro-organisms or hazardous materials, including chemicals, radioactive substance, and infectious diseases.

Disinfection / Sanitization

- **Disinfection or sanitization** : reduction in microbiological contamination by destruction or elimination of microorganisms. Saturate the cell wall and penetrate it after a certain period of time.

- **Disinfection** : The process of killing (inactivating) harmful and objectionable bacteria, cysts and other microorganisms (pathogenic) by various agents such as chemicals, heat, ultraviolet light, ultrasonic waves, or radiation.

- **Disinfecting** : The process of destruction of microorganism by use of disinfectants [A chemical or physical agent that reduces, destroys, or eliminates vegetative forms of harmful microorganisms but not spores]

Disinfection / Sanitization

- Disinfection or sanitization is never preventative
- Destroys/kills what is already there but can't prevent new microbes from growing
- Still need contamination control

Sterilization

- **Sterilization** : Complete destruction or elimination of microorganisms.

- Sterilization : A process used to render a product free of viable organisms with a specified probability. (PDA TR1)
  - SAL of 10^{-6} or 1 in 1,000,000 probability of a nonsterile unit (PNU)
  - Sterility Assurance Level : SAL

PDA TR 70

- PDA Technical Report # 70 " *Cleaning and Disinfection Programs from Aseptic Manufacturing Facilities*"
- The purpose of the cleaning and disinfection program is not only to control microbial contamination **but also** to serve as a corrective action for the loss of control for viable excursions contamination.
- While the destruction of viable cells are an integral part of the cleaning and disinfection program, the use of disinfection as a singular focus without efforts to control contamination from entering the area is without technical merit.
- Environmental monitoring (EM) evaluates the efficacy of controls on the manufacturing environment. It is through control of bioburden levels entering the area, along with cleaning and disinfection, that acceptable viable control of the manufacturing or appropriate testing environment is achieved.

PDA TR 70

- "Cleaning is a critical step in the cleaning and disinfection process because the buildup of antimicrobial chemical agent residues, product residues, particulate, and other contaminants can inhibit an antimicrobial chemical agent's efficacy."
- "Cleaning requires a nondestructive mechanical action that loosens and removes contaminants from the area or equipment surface."
- "Procedurally, a cleaning agent is applied via a nondestructive mechanical action method. Contaminates and residues are loosened and rinsed from the surface and removed with a squeegee or dry cloth."

PDA TR 70

- By lessening the level of particulates, microbes, and residues on the surface, cleaning prepares the surfaces for disinfection and the disinfection efforts become more effective because of the following:
  - " There are fewer organisms to destroy, as most have been removed from the areas."
  - "Obstructions blocking the chemical agent from contacting the organism are minimized."
  - "Chemical interference that would reduce the stability and effectiveness of the active agents is removed."
  - "Lessening of residual that can interfere with future disinfection and/or can dry or flake off and release to the environment.

Cleaning and Decontamination

- Remove materials, components, product
- Clean
- Decontaminate
- Check for cleanliness, as well as cleaning and decontamination residuals
- Visual check
- Analytical testing
  - Swab
  - Rinse

Sanitization / Surface Disinfection

- Requires approved SOP
- Use approved and qualified sanitizing agent
- Clean area to be sanitized
- Sanitize machine / line " non" product contact parts
- Sanitize walls, floors, curtains, doors, benches, fixtures, carts, etc.
- Avoid spreading contamination
- Allow for proper contact time
- Must be qualified/validated to show it is effective against anticipated microorganisms

Sterilization and Decontamination

- Moist heat
- Dry heat
- EtO (ethylene oxide)
- Gamma radiation
- Chemicals – phenols, quaternary amines
- Chloride dioxide
- Cl_2
- Hydrogen Peroxide, peracetic acid


Take a break
Coffee time

EM Risk Assessment Methods

Part I – Topic 5

Assess Activity Affecting Microbial State of Control

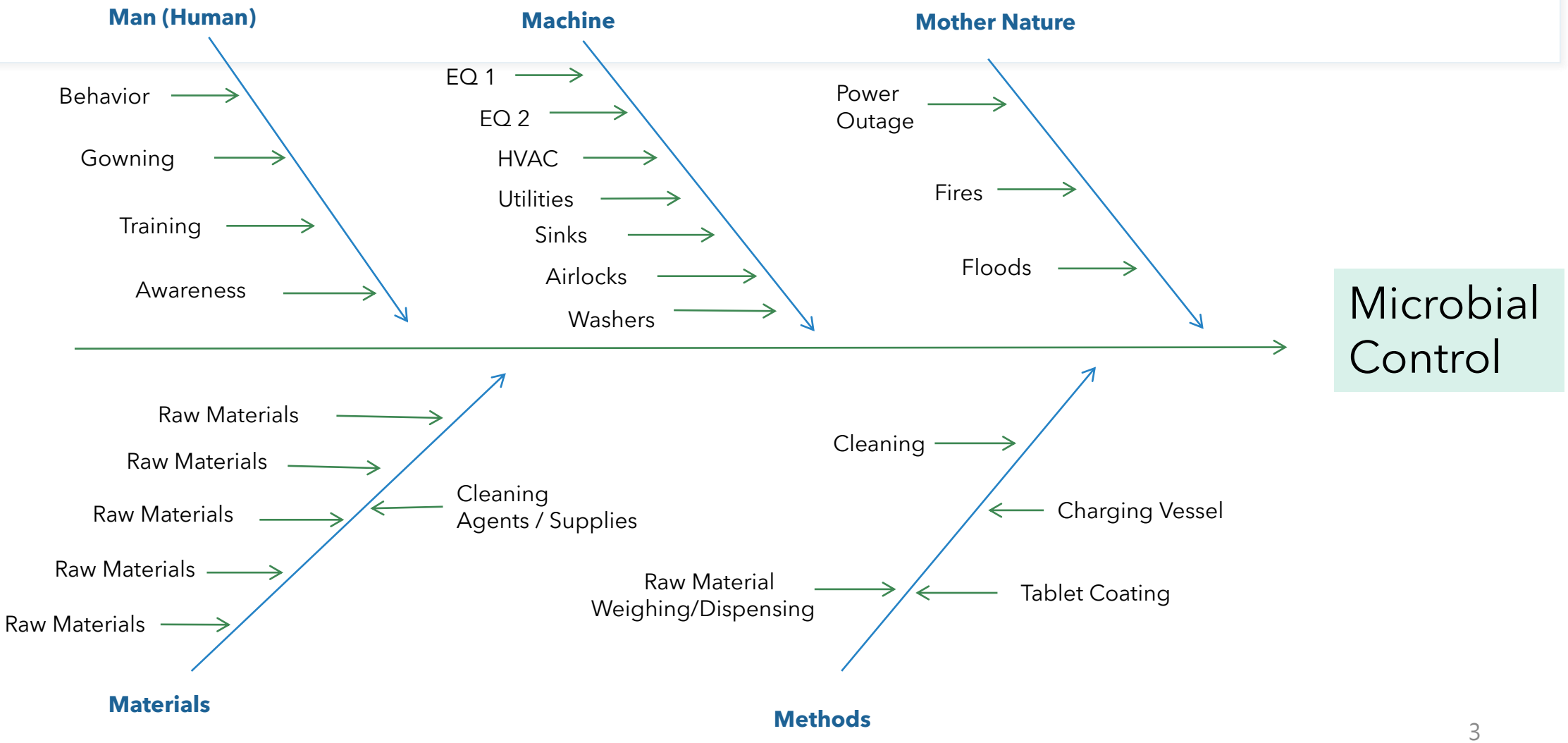
- Brainstorm
- Fishbone
- Cross functional team – Microbiologist, Manufacturing, Engineers, Quality, Facilities
  - May want to break down by sub-teams for different rooms if large facility

No need to discuss current controls in place at this point

simply identifying all areas of microbial risk

Fishbone Diagram

- Causes of Microbial Contamination



FMEA & FMECA

- **FMEA**: Failure mode and effects analysis
- **FMECA** : Failure Mode, Effects, and Criticality Analysis
- Failure Mode = how a process step can fail
- Failure effect = what is the impact if it does fail
- Criticality analysis = what is the likelihood of failure to the extent that it will result in the unwanted event

FMEA & FMECA

Ref #	Process / Unit Operation	Failure	SEV	Cause	OCC	Current Control	DET	RPR	Risk Accepted (?)	Recommended Actions	Ranking After Actions			
											SEV	OCC	DET	RPR

- SEV – Severity
- OCC – Occurrence
- DET – Detectability
- RPR – Risk Prioritization Ranking

FMEA

- Severity (SEV) : Classify the severity or importance of the effect.
- Assign points where:
  - Criteria points
    - Very low to no impact 1
    - Unimportant failure 2-3
    - Failure of medium importance may cause customer troubles 4-6
    - Critical failure, will dissatisfy customer 7-8
    - Extremely critical failure 9-10

FMEA

- Occurrence (OCC) : Estimate the probability of occurrence of the failure.
- Assign points Where :
  - Criteria Points
    - Very low probability 1
    - Failure might happen, but very seldom 2-3
    - Failure happens from time to time 4-6
    - Failure happens frequently 7-8
    - High probability that failure happens 9-10

FMEA

- Detection (DET) : Evaluate the probability of the failure detection.
- Assign points Where :
  - Criteria Points
    - Failure detection is ensured 1
    - High probability of failure detection 2-3
    - Failure detection not sure 4-6
    - Low probability of failure detection 7-8
    - Failure detection is highly improbable 9-10

FMEA

Risk Priority Number (RPN) =

Risk Priority Ranking

Severity of Failure

X

Probability of Failure Occurrence

X

Probability of Failure Detection

FMEA (example)

- The “weighted” RPN review can be summarized and addressed by suitable quality systems as follows:

1 - 125	Very low process risk	Category IV
126 - 250	Low process risk	Category IV
251 - 500	Moderate process risk	Category III
501- 750	High process risk	Category II
751 - 850	Very high process risk	Category II
851 - 1000	Extreme-critical process risk	Category I

FMEA (example)

- Mil Std. 1629A also assigns criticality for risk. We could correlate the RPN numerical assessment with the Mil Std.
- Category assignment and definition

Category I Catastrophic	A failure which can represent serious and/or unexpected product adverse experiences or serous bodily injury
Category II Critical	A failure which may cause probable unexpected product adverse experiences, severe injury or inconvenience.
Category III Marginal	A failure which may cause minor injury or inconvenience, or possible product adverse experience
Category IV Minor	A failure not serious enough to cause injury or inconvenience or other product adverse experiences.

Show Example of an EM Reassessment using FMEA & Discussion

Example of Modified Risk Assessment

- Room Number
- Room Description
- Classification
- Current Number V/NV/S location (viable/non-viable/surface)
- Microbial Risks in Rooms
- People Movements/Flow
- Number proposed New V/NV/S locations
- Comments discussing rationale based on risk

HACCP, Hazard Analysis and Critical Control Point

- The HACCP methodology for scientifically managing the microbial risk using the Clean room Contamination Control System (CCCS) is based on seven principles or steps (Whyte, 2001)

1. Identification of sources and routes of contamination
2. Assessment of the significance of the hazards
3. Identification of methods to control the hazards
4. Sampling methods to monitor the hazards and control methods
5. Establish a monitoring frequency with alert and action levels
6. System to verify contamination control system is working effectively
7. Establish and maintain documentation

- **Risk Evaluation** : A three level (high, medium, low) rating system was used to determine the overall risk prioritization rankings (RPR)

		Detection		
Occurrence		Low	Medium	High (It is not likely failure will be detected)
	High	Medium	High	High
	Medium	Medium	High	High
	Low	Low	Medium	Medium

- **Risk Identification** : For each unit operation, potential causes were identified that could lead to non-sterility
- **Risk Analysis**: For each potential failure event. Severity, Occurrence and Detection were assigned values proportional to the estimation of the risk.

Risk Category Ranking/Definition	Low	Medium	High
Severity	*N/A*	*N/A*	*Direct and severe impact to patient health; life threatening.*
Occurrence	*The possibility that the cause rarely occurs; unusual event.*	*The possibility that the cause may occur and may result in loss of sterility.*	*High possibility that the cause will occur and result in loss of sterility; a common and known event.*
Detection	*There is a high likelihood that existing controls will detect the cause or the defective product and prevent its release.*	*The cause, if it occurs, may be detected by existing controls.*	*If the cause happens, it will probably not be detected by existing controls, and defective product could be released.*

Qualitative Risk Ranking Nomenclature

Ranking	Risk Factors		
	Severity	Occurrence	Detection
HIGH	Impact of the unwanted event is severe	Occurrence is often	The process failure will almost certainly escape detection.
MEDIUM	Impact of the unwanted event is moderate	Occurrence is periodic	Controls may detect the existence of a process failure.
LOW	Impact of the unwanted event is low	Occurrence is seldom	The process failure is obvious and readily detected.

Using the Model ….Risk Prioritization Ranking

		DETECTION		
		LOW	MEDIUM	HIGH
OCCURRENCE	HIGH	This cause is likely to occur, but when it does it will be detected. If we are certain it will be detected it is low risk, but if we are not certain then it should be a Medium Risk.	This cause is likely to occur and the detection is not certain. It is a High Risk.	This cause is likely to occur and is not likely to be detected. It has a High Risk.
	MEDIUM	This cause could occur, but if it did it would be detected. Depending on the frequency of occurrence and the confidence in the detection, it is a Low or a Medium Risk	This cause could occur and it could be detected. Depending on our confidence in the detection its risk would be Medium or High.	The cause may occur and it will not be detected. This Risk is High
	LOW	This cause is not likely to occur and if it does it will be detected. This is a Low Risk.	The cause is not likely to occur and if it did it may be detected. Depending on the frequency of occurrence and confidence in detection methods, it would be Low or Medium Risk.	The cause is not likely to occur, but if it did occur it would probably not be detected. The Risk is Medium.

Note : Severity is constant “High”

HACCP Microbial Risk Assessment Example

Ref#	Process / Unit Operation	Failure	SEV	Cause	OCC	Current Control	DET	RPR	Risk Accepted (?)	Recommended Actions	Ranking After Actions			
											SEV	OCC	DET	RPR
2	Remove trays from lyophilizer, transfer trays to capper, and load trays into capper	Lack of sterility assurance	H	Stoppers are dislodged or missing	M	Procedural control for in-process visual verification of stopper presence, positioning (Qualification studies indicate this is a potential process failure)	H	H	No (The cause happens and it is not easily detected)	Add 100% mechanical stopper detection at capper in-feed (This would increase the likelihood of detection and therefore reduce the risk)	H	M	L	M

EM Program Risk-Based Approach

- Two way approach – similar but different based on prior knowledge :
  1. New EM Program
  2. Reassessment of Current/Existing EM Program

New EM program

- New Facility?
- New Controlled Environment additions to existing facility?
- Any Prior Knowledge contamination types and levels?
- Prior Knowledge is an input
- Assess Microbial Risk of New Area
- Product Microbial Risk of New Area
- Product Type
- Process

Step 1 – Planning

Risk Management Planning

- Determine Team/Members – cross functional (Micro, QA, MFG, Facilities, Engineering)
- Select Facilitator – not bias to activity
- Define Scope of Risk Activities for EM program assessment
- Determine the Risk Management Tools to be used
- Determine scoring to be used – company procedure may already exist
- Determine Communication of Risk Plan
  - Who need to be communicated to, when, how, often, how much detail

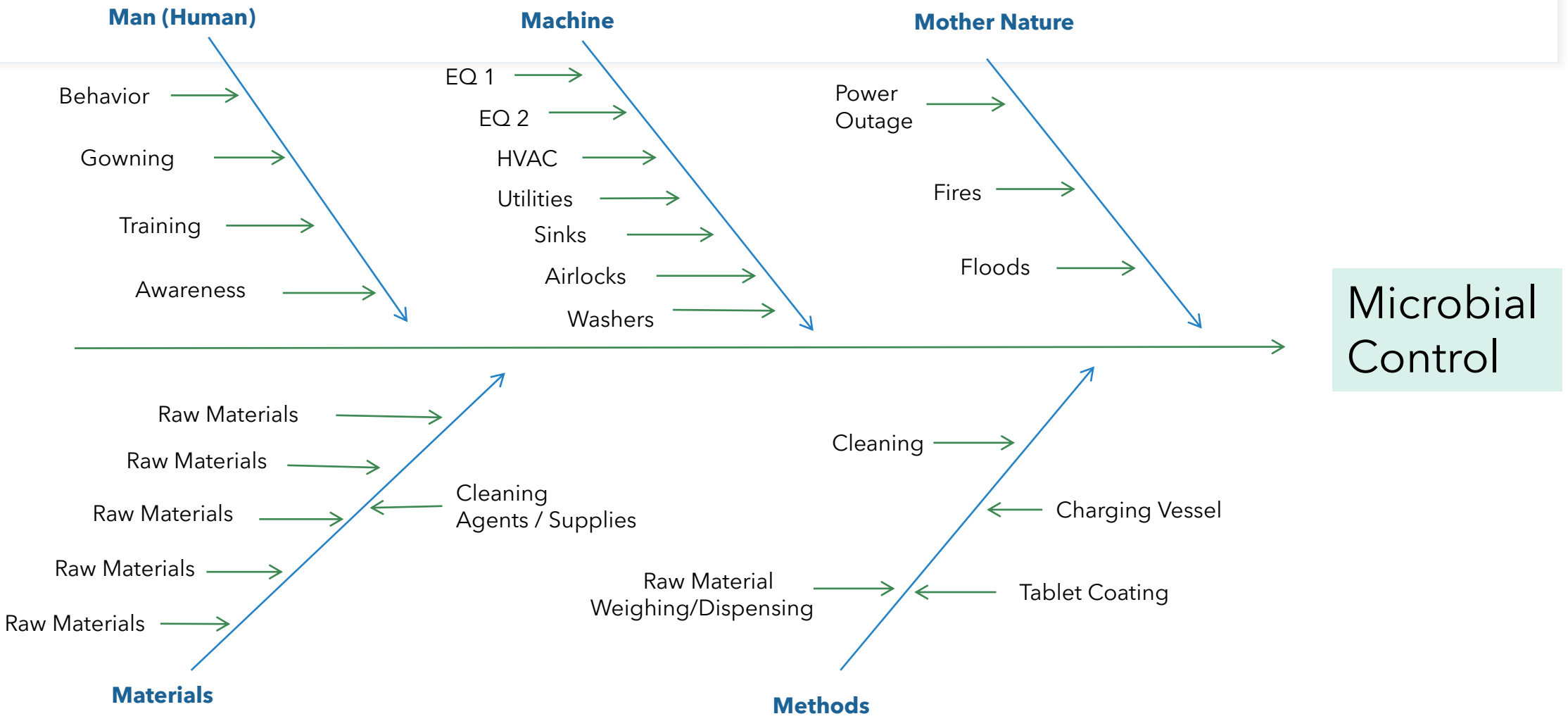
Step 2 – Assess Activity Affecting Microbial State of Control

- Brainstorm
- Fishbone
- Cross functional team – manufacturing, Engineers, Quality, Facilities
- May want to break down by sub-teams for different rooms if large facility

No need to discuss current controls in place at this point

Simply identifying all areas of microbial risk

Causes of Microbial Contamination



Step 3 – Assess Activity and Flow

- Gemba (go see) Walk each room/area if possible
  - If construction phase, do via paper until you can get in the area
  - Best case – Work with engineers prior to design and construction start to identify microbial risk points and plan them OUT
- Assess planned personnel flow
- Assess planned material flow
- Sample site locations should be based on risk of activity
- Likelihood of contamination from process
  - Open or Closed Process
  - People likely largest contributors of room contamination if closed process
- Maybe you have a wet process – Gram negatives
- Contamination from other products

Step 4 – Selection of Sample Locations and Sampling Frequencies

- Use all prior tools to now select your sample sites
- Document your rationale based on the risk of contamination
- Same size (area) and same class rooms may have different numbers of required sample sites based on risk of contamination in each room
- Make it about the activity, flow and VALUE ADDED
- **UNDERSTAND THE PROCESS IN EACH ROOM and the MICROBIAL RISK POINTS**
- Ability to assess effectiveness of sanitization – for that reason floor and wall locations may still be needed in your program – reduced number and justification/through process

Step 5 – Perform EM-REM

- Next use the information obtained in the brainstorming activity and floor assessment as a foundation to perform a risk assessment
- Include known controls and risk mitigations
- Cross functional team – Microbiologist, Manufacturing, Engineers, Quality, Facilities
- EM REM

Reassessment of Current EM Program

- Year of DATA which is KNOWLEDGE which you will use to make changes to your existing EM program
- Sample site locations
- Frequency
- Type of media
- Incubation

Step 1 – Planning

Risk Management Planning

- Determine Team/Members – cross functional (Micro, QA, MFG, Facilities, Engineering)
- Select Facilitator – not bias to activity
- Define Scope of Risk Activities for EM program assessment
- Determine the Risk Management Tools to be used
- Determine scoring to be used – company procedure may already exist
- Determine Communication of Risk Plan
  - Who need to be communicated to, when, how, often, how much detail
- Impact to Regulatory Filings
- Cost Impact

Step 2 – Previous Risk Assessment Review

- Do you have a Product and/or Process Risk Assessment which already identifies the microbial contamination risk points in your processes?
  - HOPEFULLY – YES
  - *If No- This is your step 2*, Q8/Q9/Q10 or ISO 14197
  - Cross-functional activity
  - Fishbone from New EM Program Slides
- Assume Yes
  - Perform a review of this document to identify your microbial risk points as a starting place

Step 3 – Data Review Current EM

- Assess current EM locations and results
- What are your trouble spots – frequent alerts/actions or recovery of objectionable organisms
- Flag these – likely keep for your new EM program

Step 4 – Floor Walk/Gemba

- Assess Eqmt, material and people flow ON THE FLOOR
- Talk to current Production Operators
- Ask them what they see as microbial risk points
- They have VAST knowledge from being on the floor everyday
- Cross functional – smaller group then risk assessment
- Microbiology Lead
- MFG support
- Most process knowledge
- Where have you had bioburden or water or compressed air contamination concerns?

Step 5 – Select New Sample Locations and Sampling Frequencies

- Document all findings and risk points
- Perform EM-REM – using knowledge gained in product and process risk assessments
- Talk about activity, people and flow in each room
- Rationalize new chosen sample locations based on microbial contamination risks in these areas
- Documentation needs to be a living document, signed off Site Head, QA Head, Microbiology Management at a minimum.

EM – REM

Environmental Monitoring Risk Evaluation Model

Evaluate if current samples are risk-based or not –
rationalize risk-based sample site selections

Risk Based EM – EM-REM

- ***Risk*** *– combination of the impact of the hazard or unwanted event and its likelihood of occurring and harming the patient*
- Focus on proximity of sample location to potential contamination
- Severity of product contamination is always HIGH risk
  - Consider this a constant
  - Remove from assessment
- Focus on product and process knowledge, material flow, people flow, duration of time that people in working in the area

Risk Based EM – EM-REM

- Use Key Word risk assessment approach instead of using general, subjective terms for the level of risk (often, frequently, rarely)

- Example : Duration of activity:
  - Long, Medium, Short – subjective
  - > 1 minute – objective, measurable

EM – REM , Proximity

- How close the EM sample is to area of potential contamination

Risk Level	Proximity
High	EM sample is in immediate proximity to a potential contamination source, open product/processing – microbial contamination source (0-2 feet away)
Medium	EM sample is near the potential microbial contamination source but not immediately proximal (3 feet to 5 feet away)
Low	EM sample is not near a potential microbial contamination source or open product/processing (5 or more feet away)

EM – REM , Number of PPL Routinely Present in Area

- The more people in the areas of the sample location, the higher the risk of contamination
  - The longer the activity, the more risk for contamination
  - Traffic Flow – high, medium, low
  - Number of people – more ppl, more risk
  - Utilize batch record data, talk to operators, time the actual events to determine and set up a scale for your own EM risk assessment

Risk Levels	Number of People Working Near Sample Site
HIGH	8 or more people
MEDIUM	2-7 people
LOW	0 or 1 person

EM – REM , Duration – Time,

- The longer the activity, the more risk for contamination

Risk Levels	Time People are in Location
HIGH	> 6 – 8 /10+ hours (Entire Shift)
MEDIUM	>1- 6 hours
LOW	Less than 1 hour

Risk Based EM

- Two Level Risk Block Assessment Method:
- Used to consider relationship of all three-risk elements
- Two three block risk tables
  1. Duration/Time and number of People – to provide a risk level
  2. Risk Class and Proximity
- Determine if sample location is high, medium or low risk
- Goal – select and rationalize risk-based sample locations as part of your EM Program (ensure high risk locations have sample sites)

EM – REM

- EXAMPLE 1:
- Cell Culture Room, Viable Air Sample V1, Center of Room, Location Not Near Processing Activity

Factor	Result	Risk Level
# PPL	5	MEDIUM
Duration / Time	>1 hour	LOW
Proximity	Not near any processing, ppl or contamination sources	LOW

EM – REM

- Duration – Number of People Risk Class Determination

Duration (Time)

Number of people

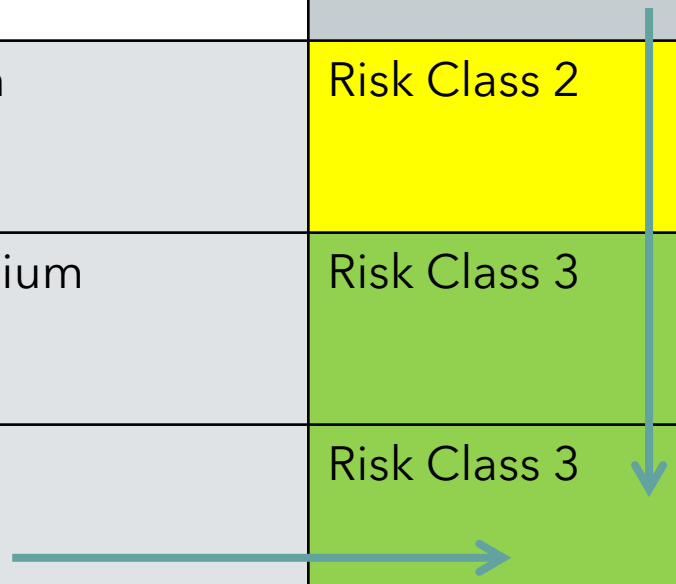
	Low	Medium	High
High	Risk Class 2	Risk Class 1	Risk Class 1
Medium	Risk Class 3	Risk Class 2	Risk Class 1
Low	Risk Class 3	Risk Class 3	Risk Class 2

EM – REM

- Proximity – Risk Class Comparison Table

Proximity

Risk Class (From Table Above)	Low	Medium	High
High	Risk Class 2	Risk Class 1	Risk Class 1
Medium	Risk Class 3	Risk Class 2	Risk Class 1
Low	Risk Class 3	Risk Class 3	Risk Class 2



This is a **LOW** risk sample location

Risk Based EM

- EXAMPLE 2:
- Floor RODAC F2 – Next to Floor Drain in Wash Room

Factor	Result	Risk Level
Duration / # PPL	8	HIGH
Time	3 hour	MEDIUM
Proximity	Next to drain	HIGH

EM – REM

- Duration – Number of People Risk Class Determination

Duration (Time)

Number of people

	Low	Medium	High
High	Risk Class 2	Risk Class 1	Risk Class 1
Medium	Risk Class 3	Risk Class 2	Risk Class 1
Low	Risk Class 3	Risk Class 3	Risk Class 2

- Proximity – Risk Class Comparison Table

Proximity

Risk Class (from Table above)	Low	Medium	High
High	Risk Class 2	Risk Class 1	Risk Class 1
Medium	Risk Class 3	Risk Class 2	Risk Class 1
Low	Risk Class 3	Risk Class 3	Risk Class 2

This is a **HIGH** risk sample location

Example

Airlock ot Gowning Room 1234								
Potential Sources Micro Contamination	Sample location	Sample ID	# of People	Duration/ Time	Risk Class	Proximity	Risk Priority of Sample	Comments
People, high traffic flow, ingress if breach from CNC hall	Insider door from CNC to PAL in front of door	V1, NV1, F1	M	L	3	H	2	#P:Max number ppl one time will be 4
Ceiling return vent area, Near door to CNC hall, people, high traffic	Wall across from door, under ceiling return vent	W1	M	L	3	H	2	Ceiling return vent and high traffic flow are
People, traffic flow	Wall to locker room	W2	M	L	3	M	3	Assess wall cleaning
People, materials, traffic flow, cart wheels	In front of shelf to gather gowning materials and gown in room door	V2, NV2, F2	M	L	3	H	2	Highest traffic flow area
People touching door	Handle to gown room door	D1	M	L	3	H	2	All people must open door with handle
People touching door	Door (glass surface)	D2	M	L	3	H	2	People may touch glass when entering room

Risk Based EM

- Opportunity to evaluate the risk and consider risk reduction for high risk locations that score HIGH or maybe even MEDIUM risk
  - Evaluate changes to process to reduce the risk of the location
- Risk assessment allows for training of operator on high risk areas in the room
- Use risk assessment to evaluate overall process and determine priorities in implementing changes to the process
- Allows for identification of high risk locations to rationalize EM locations
- Now need to tie in controls to select sample locations

Risk Based EM

Conclusion

- Risk assessment in EM is a tool to gain understanding of the high risk locations in each room
- Objective is :
  - To gain knowledge so you can make informed decisions about process and select risk-based EM sample locations
  - Determine where to improve the process and reduce risk
- **Control of risk = good process design**
- Good process design begins with a firm understanding of the process


TIME FOR
A BREAK!

EM Equipment

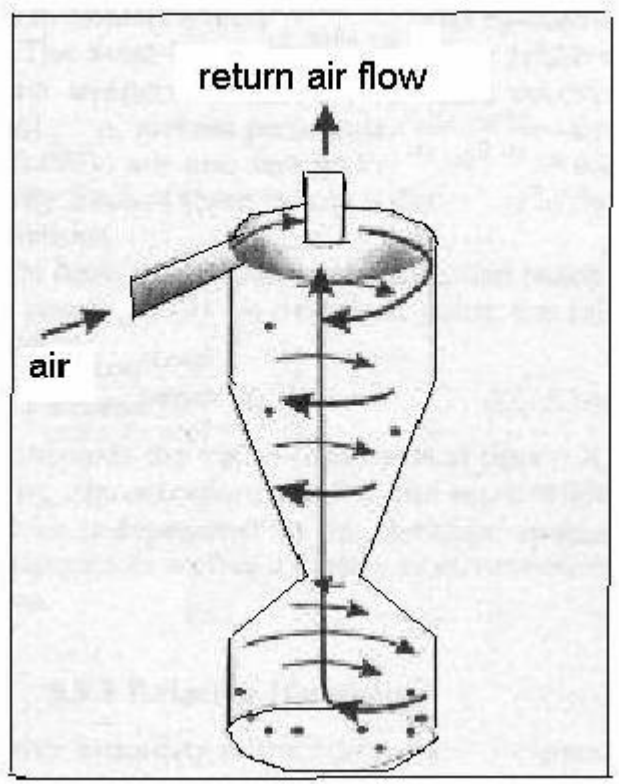
Part I – Topic 6

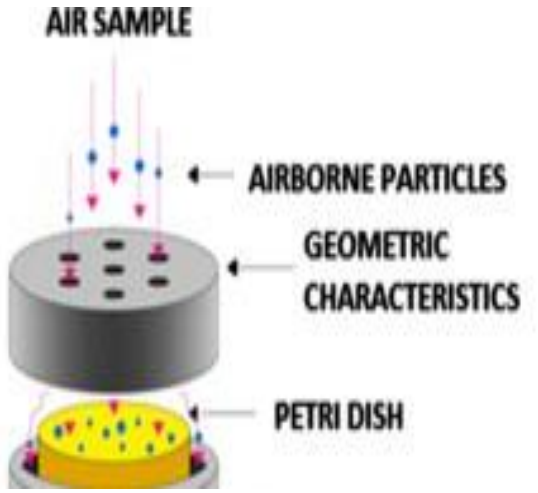
Portable Viable Air Samplers

- Sieve Impaction (many device)

Portable Viable Air Samper

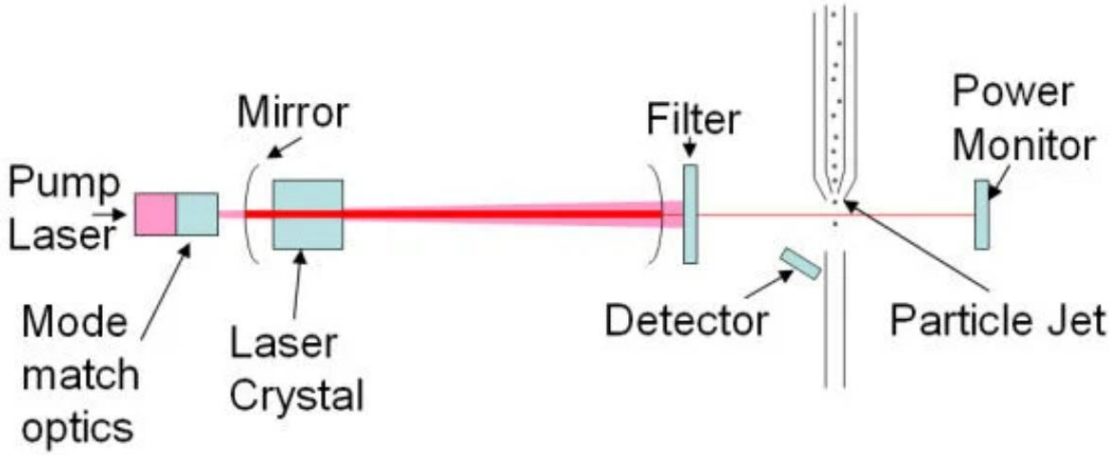
- Centrifugal Air Sampler (RCS)

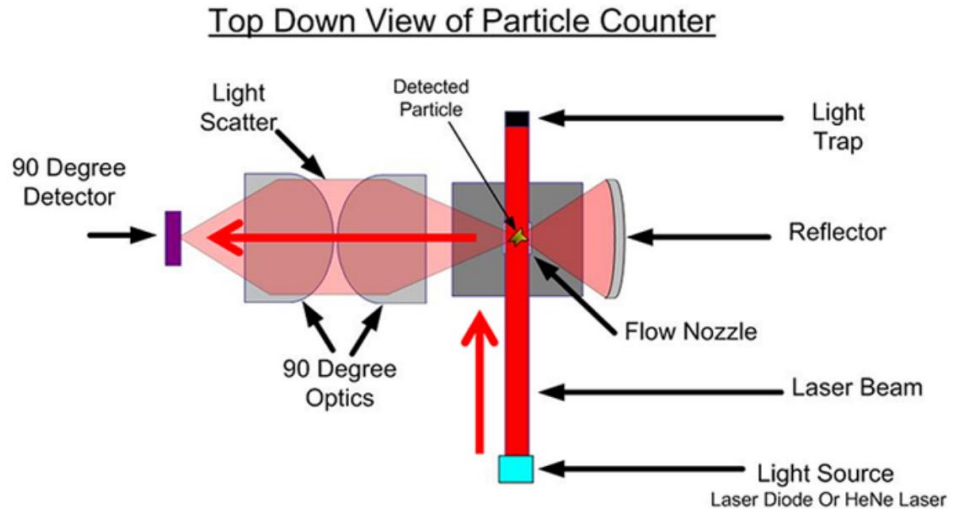




Non-Viable Particulate Monitors

Viable vs. non-viable counter





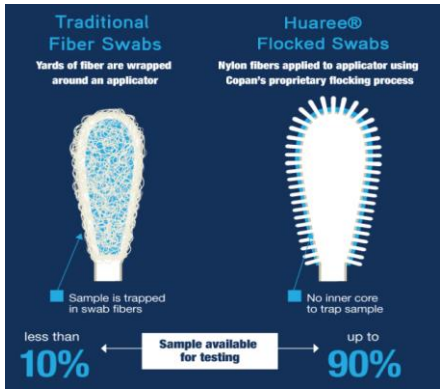
Surface Monitoring

Contact Plates

- Contact plates (RODAC)
- Use on flat surfaces
- Contact plates can offer "better recovery" than swabs and utilized more often (where surface and locations permits)
- Neutralizer in media
- Sampling done on equipment, work surfaces, floors, walls and product contact surfaces **after processing is complete!**

Swabs

- Swab recovery studies
- Contact plates can offer "better recover" than swabs and utilized more often (where surface and location permits)
- Flocked swabs offer "better recovery" than spun/cotton swabs due to physical composition
- Qualitative presence/absence test for microbiological contamination or pour plate/filter
- Use on irregular surfaces



Flexible Films

- Similar to contact plates
- Use on flat surfaces
- Neutralizer in media
- Sampling done on equipment, work surfaces, floors, walls and product contact surfaces **after processing is complete!**

Personnel Monitoring

- Certification
- Recertification every Six months
- Post BSC plating

Viable Air

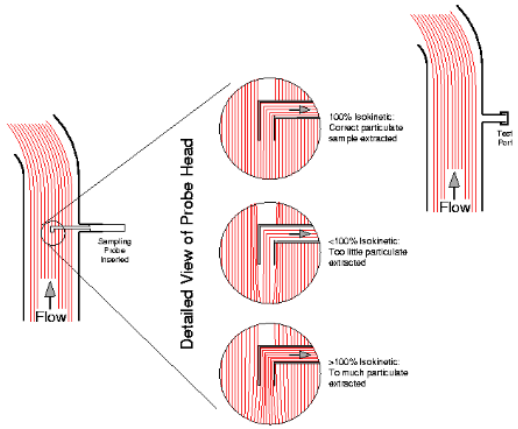
- Active versus passive *(next slide settle plates)*
- Volume – 1 cubic meter or less
- Continuous
- Reporting Results Liter or Cubic Meter

Settle Plates

- Use Deep Fill 30 mL+ media to avoid desiccation
- Thoughts versus Active??

Particulate Monitoring

- Discrete laser particle monitoring
- Particle size ≥ 0.5µm and/or ≥ 5µm
- Grade A continuous set up through end of process
- Within 1 foot of critical process
- Sample rates cfm or critical process
- Sample volume 1 cubic meter or less
- Fixed for Grade A/BSC, portable routine
- Isokinetic probes/sample heads
- Tubing kinks in manifolds



Rapid Micro for EM

- Growth Direct
- Biovigilant IMD-A
- TSI BioTrak
- PMS BioLaz
- Biomerieux Scan RDI

EM for Different Manufacturing Processes

Aseptic Processing

- Highest number of sample locations and highest monitoring frequency
- Follow regulatory guidance
- USP <1116>
- Isolator/RABS EM design
- Personnel EM
- Continuous nonviable
- Swabs and Contact Plates post processing
- EM part of batch record
- Risk Assessment

Non-Sterile

Fewer Guidance Available

USP <1115>

Rely on Risk Management

Low Bioburden

Fewer Guidance Available

USP <1115>

Rely on Risk Management

Cell and Gene Therapy/ATMP

- Mix of low bioburden and aseptic processing
- Little regulatory guidance
- USP <1116> for aseptic parts
- Isolator/RABS EM design
- Personnel EM
- Continuous Nonviable
- Swabs and Contact Plates post processing
- Open Processing
- Plasmids/Viral Vectors
- Risk Assessment **VERY** important

Terminal Sterilization

- Ensures that the spore (heat resistant) bioburden levels presented to the product.
- Sterilization cycle does not exceed the validated capabilities of the process and that the desired sterility assurance levels are achieved (SAL 10^{-6})
- Bioburden still must be controlled in your process
- Gamma resistance microbes


Break Time

EM Trending and Alert Action Level Setting

Part I – Topic 7

Alert and Action Levels

- Typically Action Levels are set based on Regulatory Guidance
- Alert Levels should be set based on historical data

Guidance Particle Levels

Prticle Size	ISO 14644	US FDA (Aseptic Processing Guidance)	USP <1116>	WHO	Japan (Aseptic processing Guidadnce)	JP XVI
	ISO 5	ISO 5/Class 100	ISO 5/Class 100	Grade A Grade B (at rest)	Grade A Grade B (at rest)	Grade A Grade B (at rest)
≥ 0.5 µm	3520	3520	3520	3500	3520	3520
≥ 5 µm	29	Not specified	Not specified	1 cubic meter	20	Not specified
	ISO 6	ISO 6/ Class 1000	ISO 6/Class 1000	NA	N/A	N/A
≥ 0.5 µm	35,200	35,2000	35,200	NA	N/A	N/A
≥ 5 µm	290	Not specified	Not specified	NA	N/	N/A
	ISO 7	ISO 7/ Class 10,000	ISO 7/Class 10,000	Grade B (operation) Grade C (at rest)	Grade B (operation) Grade C (ar rest)	Grade B (operation) C (at rest)
≥ 0.5 µm	352,000	352,000	352,000	352,000	352,000	352,000
≥ 5 µm	2,900	Not Specified	Not specified	2,000	2,900	Not specified
	ISO 8	Class 100,000	ISO 8/Class 100,000	Grade C (operation) Grade D(at rest)	Grade C(operation) Grade D(at rest)	Grade C (operation) Grade E (at rest)
≥ 0.5 µm	3,520,000	3,530,000	3,520,000	3,520,000	3,520,000	3,520,000
≥ 5 µm	29,000	Not specified	Not specified	20,000	29,000	Not specified

Max permitted total particle concentration for classification

Grade	Maximum limits for total particle ≥ 0.5 um /m^3		Maximum limits for total particle ≥ 5 um /m^3	
	At rest	In operation	At rest	In operation
A	3 520	3520	Not specified (a)	Not specified (a)
B	3 520	352000	Not specified (a)	2 930
C	352 000	3 520 000	2 930	2 930
D	3 520 000	Not predetermined (b)	2 930	Not Predetermined(b)

(a) Classification including 5 um particles may be considered where indicated by the CCS or historical trends
(b) For grade D, in operation limits are not predetermined. The manufacturer should establish in operation limits based on a risk assessment and routine data where applicable.

Max permitted microbial contamination level during qualification

Grade	Air sample CFU/m ^{3}	Settle plates (diameter 90 mm) CFU/4 hours	Contact patles (diameter 55 mm) CFU/plate
A	No growth		
B	10	5	5
C	100	50	25
D	200	100	50

Max permitted total particle concentration for monitoring

Grade	Maximum limits for total particle ≥ 0.5 um /m^3		Maximum limits for total particle ≥ 5 um /m^3	
	At rest	In operation	At rest	In operation
A	3 520	3520	29	29
B	3 520	352000	29	2 930
C	352 000	3 520 000	2 930	2 930
D	3 520 000	Not predetermined	2 930	Not Predetermined

Max action limits for viable particle contamination

Garde	Air sample CFU/m^3	Settle pates (diam. 90 mm) CFU/4 hours	Contact plates (diam. 55mm) CFU / plate	Glove print, Including 5 fingers on both hands CFU/glove
A	No growth			
B	10	5	5	5
C	100	50	25	--
D	200	100	50	--

Alert and Action Levels

- Levels need to be established
- Documented in an SOP
  - How to respond when exceeded
- Reviewed periodically and adjusted if needed
  - Routine Trend Analysis
- Regulatory Guidance or compendial levels always supersede setting your own levels

Setting Alert and Action Levels

Cut-off Value Approach

- All the test data for a particular site, or group of similar sites, are arranged in a histogram and the alert and action levels are set at values whose monitoring results are responsively 5% and 1% higher than the level selected.
- Other percentiles may be used in establishing levels. A variation is to take the last 100 monitoring results and use the 95th and 99th percentile values as the alert and action levels.

Setting Alert and Action Levels cont'd

Normal Distribution Approach

- The mean and standard deviation of the data are calculated, and the alert and action levels are set at the mean plus two(2) and three(3) times the standard deviation, respectively.
- This approach is best used for high counts and when the data is normally distributed only
- A Poisson Distribution is used for low counts.

Setting Alert and Action Levels cont'd

- Environmental Monitoring data is usually not normally distributed.
- A non-parametric Tolerance Limits approach to setting alert and action levels should be used.
- Allow us to assert with confidence at least 95% (K=0.95) that 100(P) or 99% of a population lies below the value
- For Distribution-Free Tolerance Limits, Minimum Sample Size are N=60 for 95/95 (Alert Limit) and N=300 for 95/99 (Action Limits) (PDA TR 13)

Setting Alert and Action Levels cont'd

- Contamination in pharmaceutical cleanrooms does not fall within a normal distribution
- Environmental monitoring data should be evaluated to determine the most suitable approach to level setting.

Trending EM Data

- Why Trending?
  - Confirm you are meeting your set alert and actions level
  - Shows your contamination control is working
  - Warns of a drift from control
  - React before out of control

Trending EM Data cont'd

- Focus on trends, not single events
- Snapshot over time
- How often should you trend?
  - Daily, Weekly, Monthly, Quarterly, Annually

Trending Tools

- Range from very manual to very custom electronic specific systems
  - Spreadsheet (Excel)
  - LIMS System
  - EM Specific Software
    - NOVA-EM
    - MODA-EM
- Choose what is right for your company!!!

Trending EM Data

- Know your Audience
  - Microbiology Department to understand data
  - Site Level
    - Manufacturing Rooms
    - Personnel
    - Maybe Daily, Weekly, Monthly

Trending EM Data cont'd

- Know Your Audience
  - FDA
  - Management Review
  - Corporate High Level
    - Hight Level Trends
    - Maybe Quarterly Excursion Rates
    - Could only be one or a few quality metrics

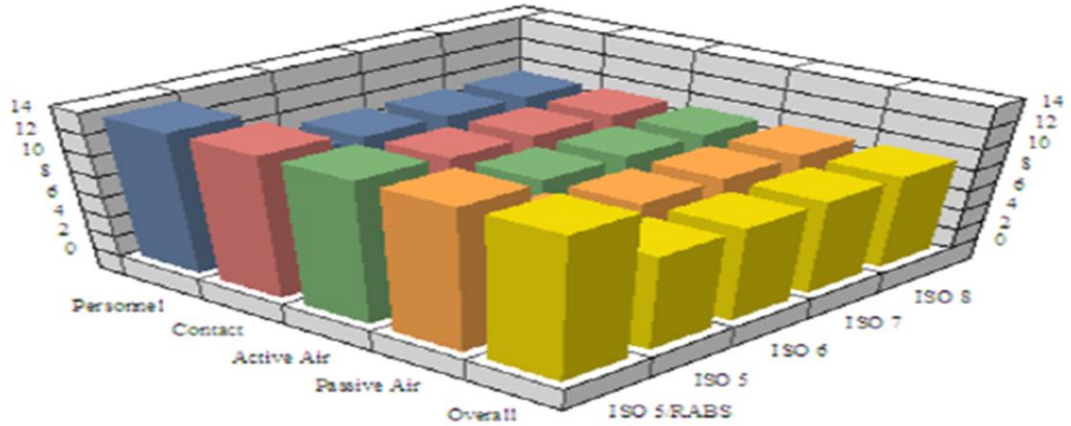
Contamination Recovery Rates for USP <1116>

Room Classification	Suggested initial Contamiantio Recovery Rate (%)			
	Active Air Sample	Settle Plate (9 cm) 4-hour Exposure	Contact Plate or Swab	Glove or Garment
Isolator/closed RABS or ISO 5 better	<0.1	<0.1	<0.1	<0.1
ISO 5	<1	<1	<1	<1
ISO 6	<3	<3	<3	<3
ISO 7	<5	<5	<5	<5
ISO 8	<10	<10	<10	<10

Contamination Recovery Rates for USP <1116>

Recovery Rate Report

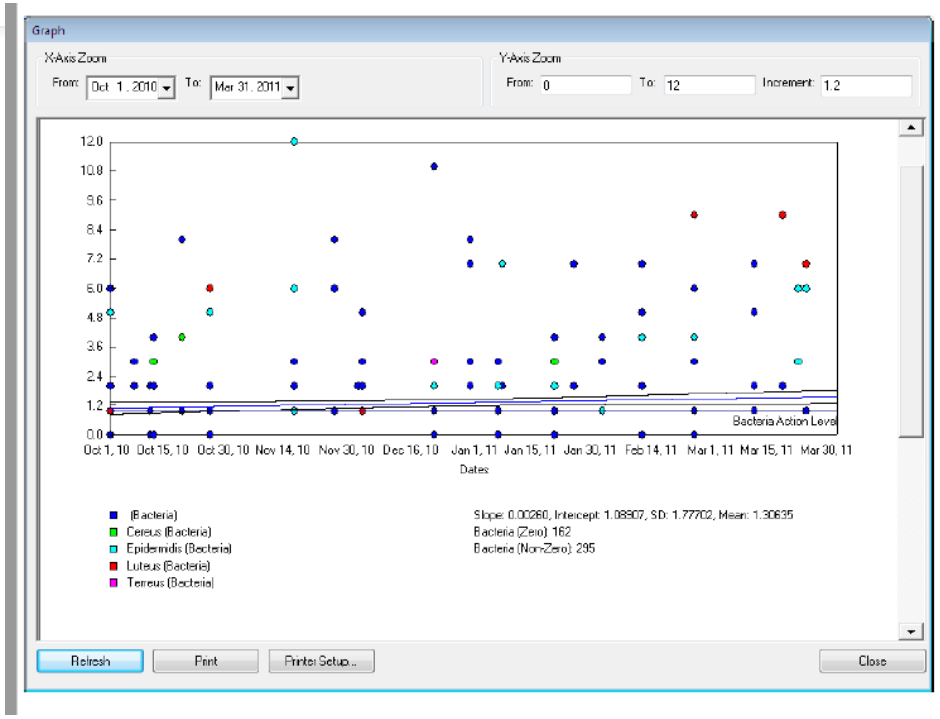
Filter by:
Building = Building 1
Shift = 1
Summary Date = From 6/1/2010 to 6/1/2012



Classification	Active Air (%)	Passive Air (%)	Contact (%)	Personnel (%)	Overall (%)
ISO 5/RABS	13.64	13.64	13.64	13.64	13.64
ISO 5	9.09	9.09	9.09	9.09	9.09
ISO 6	9.09	9.09	9.09	9.09	9.09
ISO 7	9.09	9.09	9.09	9.09	9.09
ISO 8	9.09	9.09	9.09	9.09	9.09

NR: No Recovery Performed

Trending Microorganisms



- Automated Systems allow for microorganism identification and analysis
- Identify your microorganisms and store pictures with your identification
- View the distribution of your genus, species, strain etc.
- View the distribution by room, department, etc.
- Trend microorganisms detected in a specific areas or correlate with other environmental information

Trending Microorganisms cont'd

EM Investigation

EM Investigations

- Investigations and corrective actions are needed in response to :
  - An action level excursion
  - An adverse trend
- Determine a cause and effect relationship (i.e., sources of contamination)
- Corrective action steps should be pre-specified in a written plan for consistency
- The written plan should define the level of investigation required if there are multiple or sequential excursions

EM Investigations cont'd

- Part of the investigation should include product impact assessment
- Evaluate risk to other products manufactured in the same time frame
- Start with Microbial Identifications
  - Or start investigation but get ID info ASAP

Room Air Excursion – What to Look At?

- Review level of personnel activity
- Review aseptic technique of personnel
- Review training records
- Review gowning procedures and equipment for area
- Review room disinfection/sanitization procedures, sanitization intervals, disinfectant efficacy
- Review training records of individuals performing sanitization/disinfection
- Review / perform air flow patterns/HEPA integrity tests
- Review trends and any possible incidents of HVAC outages
- Inspect incoming air filters for leaks and pressure differential across filter
- Check area pressure differentials
- Review relevant, recent data at the same sites and subsequent monitoring results

Surface Viable Excursion – What to Look At? Cont'd

- Review room disinfection/sanitization procedures, sanitization intervals, disinfectant efficacy
- Review training records of individuals performing sanitization/disinfection
- Review level of personnel activity
- Review videos if available
- Review training records
- Review gowning procedures and reequipments for area
- Room damage – paint, chip, leak, pitting
- Production Activities
- Potential Product Impact

Gowning EM Excursion – What to Look At? Cont'd

- Operator interview
- EM trend data on person(s)
- Room EM data
- Review videos if available
- Review training records
- Review gowning procedures and requirements for area
- IPA/glove sanitization procedures
- Isolator or RABS gloves
- Pinhole leaks
- Leak test results
- Material transfer
- Production Activities
- Potential Product Impact

Risk Analysis

- Fishbone Diagram
- 5 Why's
- Show you thought of all possible root causes
  - Narrow down to most probable 1 or 2

Detailed Report

- Document Findings in Formal Report
- Approvals in Doc Control system
- Per SOP requirements

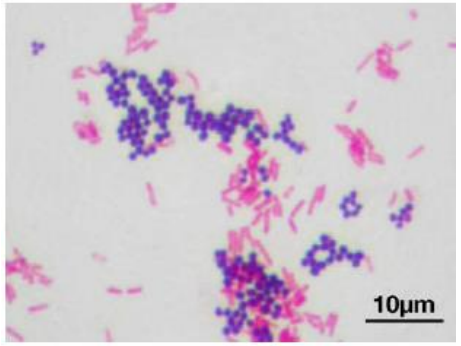

Break Time

Microbial Identifications

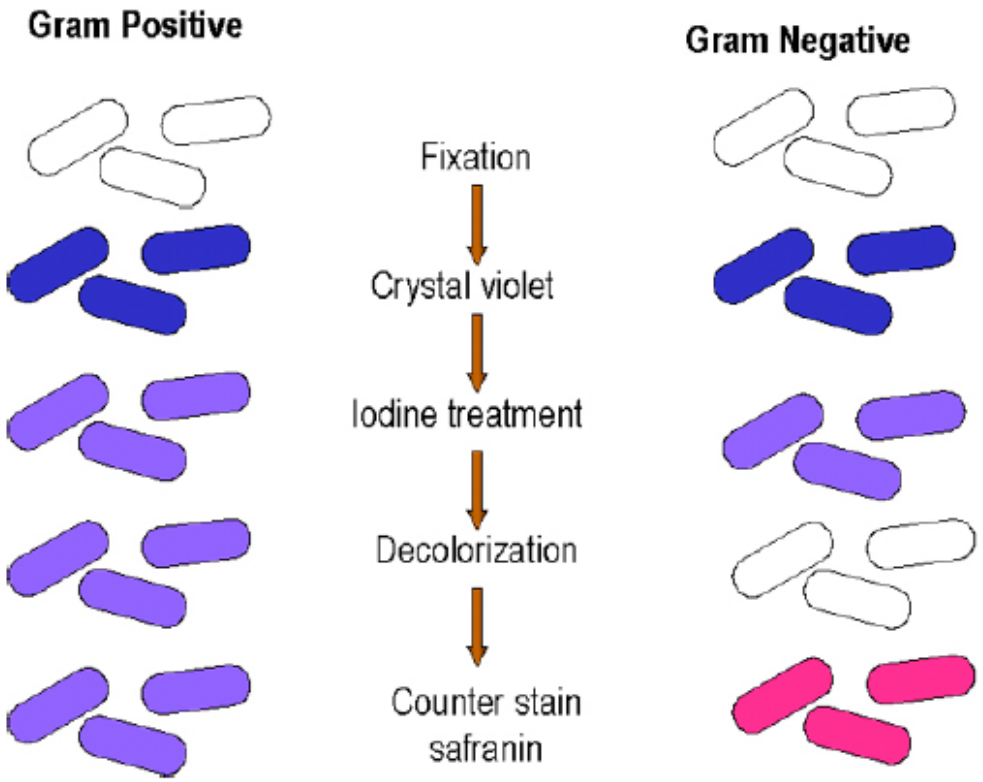
Part I – Topic 8

Gram Positive and Gram Negative Bacteria

- Gram stain is used to differentiate and identify bacteria by type
- Gam positive stain purple
- Gram negative stain pink
- Gram variable – both
- **First step in identification of bacteria**



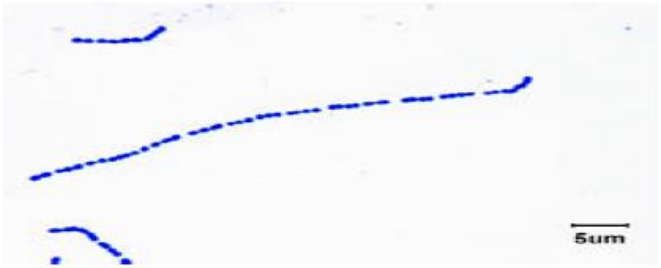
Gram Stain


Gram Positive
Fixation
Crystal violet
Iodine treatment
Decolorization
Counter stain
safranin
Gram Negative

Bacteria

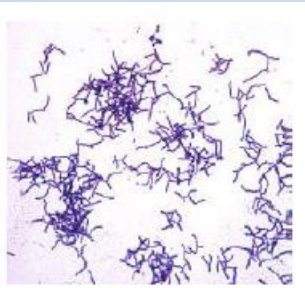
Gram Positive Cocci

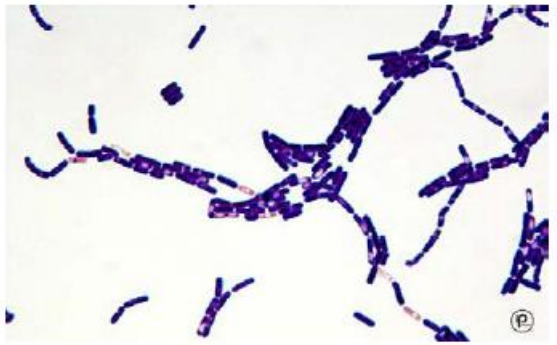
- Skin organisms
- Shed off in clean rooms
- Most prevalent microbe

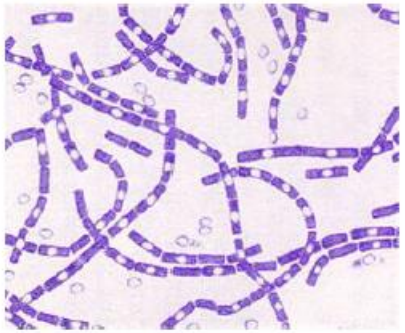


Bacteria

Gram Positive Rods





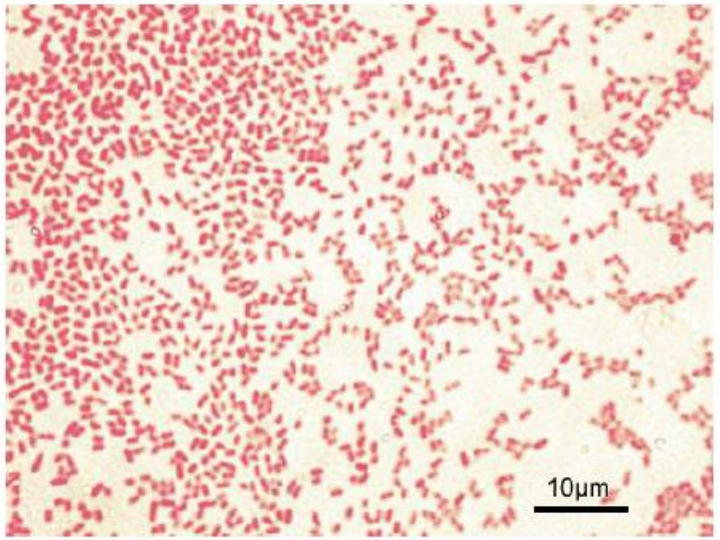


- Soil Organism
- Environmental isolates
- Spore Forming
- Require consideration when selecting cleaning agent

Bacteria

Gram Negative Rods

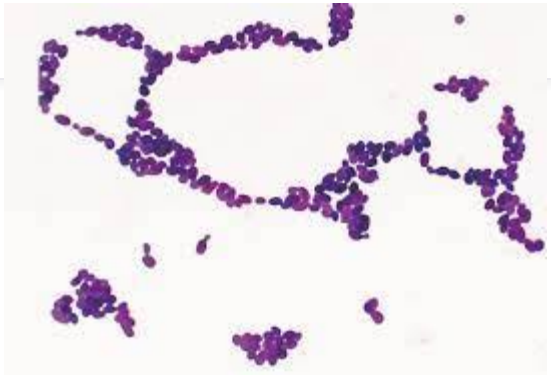
- Water organisms
- Gastrointestinal



Fungal , Yeast and Molds

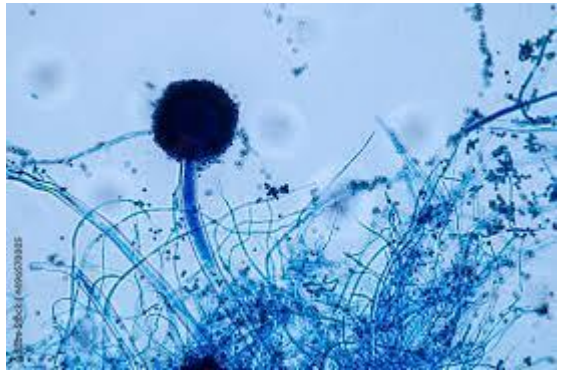
Yeasts

- Small, single celled plants
- Feed on sugars and starches
- *Candida*



Molds

- Plants
- Grow in air, moisture
- Produce spores, abundant in the air
- *Aspergillus*
- *Penicillin*



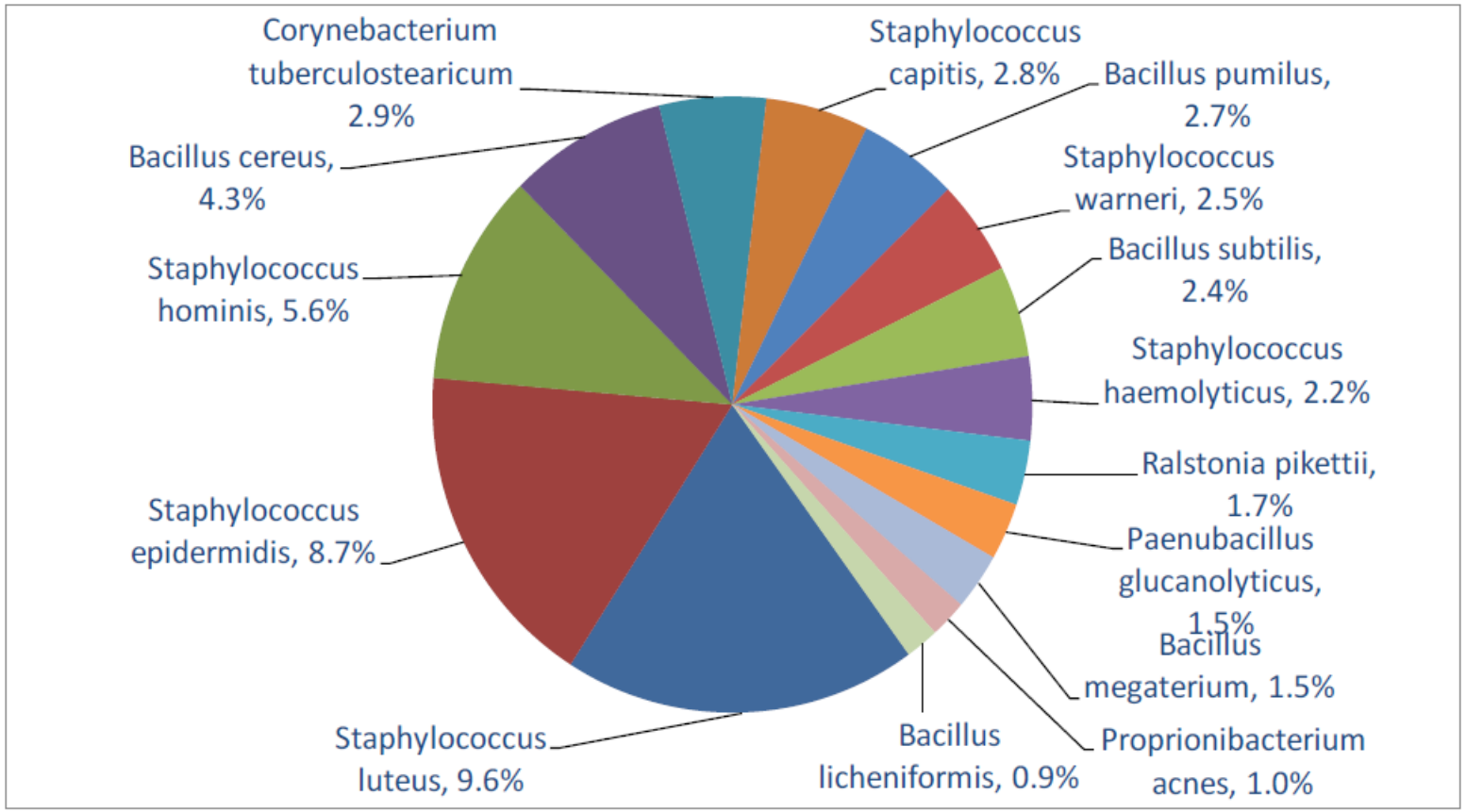
Pathogenic Microorganism

- Aseptic = absence of the potential to cause infection
  - In aseptic processing, we are concerned with any microbial contamination
  - Trying to avoid pathogenic organisms from harming patients
  - **Non-pathogenic** – most common, non disease causing
  - **Opportunistic pathogens** – cause disease under appropriate conditions
    - Need a path of entry (open wound, weak immune system)
  - **Obligate pathogens** – cause disease on their own, bacteria must infect a host to survive
- Sterile = free of microorganisms

Microorganism Identification

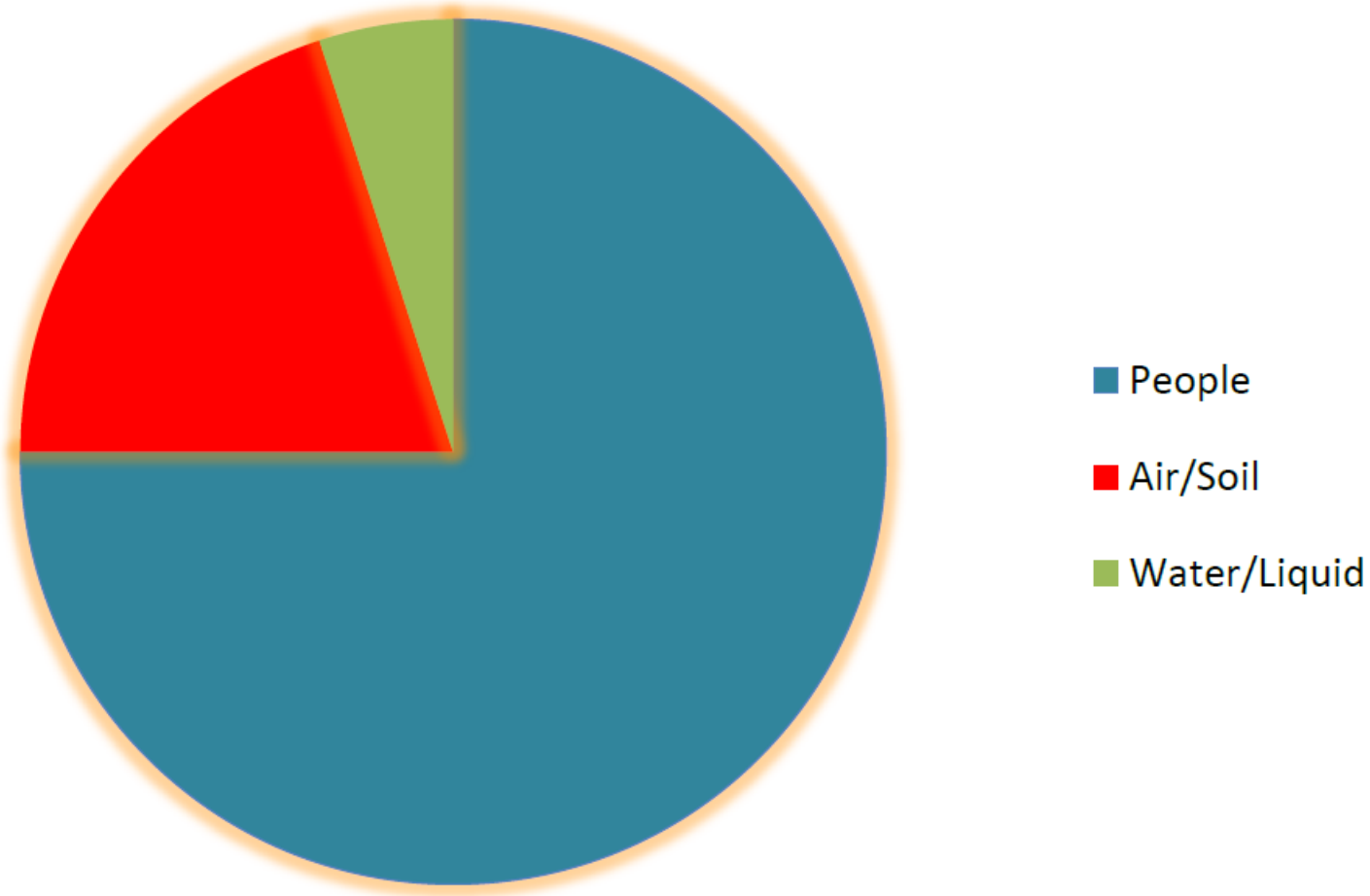
- Gram Stain
- Biochemical (Selective Assays)
- Coagulase Test
- Oxidase Test
- Catalase Test
- Indole Test
- Phenotypic Identification (incorporates reactions to different chemicals or different biochemical markers)
- Genotypic Identification
- Proteomic

Microorganism Common IDs



Source : Bacteria Most Submitted for Identification Testing During 2010, Barry A. Friedman, Posted May 17, 2011

Distribution of Microbes



Source : Bacteria Most Submitted for Identification Testing During 2010, Barry A. Friedman, Posted May 17, 2011

References for EM overview

- Annex 1
- FDA Aseptic Processing Guidance
- ISO 14644-1, -2
- PDA TR 13
- PDA TR29
- PDA TR70
- USP 1115
- USP 1116
- EU ATMP Guidance
- PDA microbiology workshop presentation

End of Part I