Centre for Biopharmaceutical Excellence

Aseptic Processing Practices and Process Validation of Aseptic Operators

Module Outcomes

On completion of this module the participant should be able to:

- Relate relative risks between terminal sterilisation and aseptic processing
- Interpret the requirements of the FDA and PICs guides to aseptic processing.
- Define the importance of media fills/process simulations to sterility assurance
- State the validation requirements and acceptance criteria for aseptic media fills
- Identify “worst case” conditions and critical interventions

Module Topics

- Describe risks associated with aseptic processing
- Sterile Processing GMP Expectations
- Identify related support systems
- Outline a worst case media fill for aseptic processing validation

Important References

- PIC/S Guide to Good Manufacturing Practice for Medicinal Products Annex 1 Manufacture of Sterile Medicinal Products
- PIC/S Recommendation on the Validation of Aseptic Processes January 2011
- FDA Guideline on Sterile Drug Products Produced by Aseptic Processing Sept 2004
- PDA - Points to Consider for Aseptic Processing
- ISO 13408-1:2008 Aseptic processing of health care products – Part 1: General requirements (parts 2-8 also deal with aseptic processing)
- PDA Technical Report No. 28 Process Simulation Testing for Sterile Bulk Pharmaceutical Chemicals

How do we make sterile products ?

There are two main approaches used in our industry

- **Terminal sterilization** – where the **final filled product is sterilized** (e.g. in an autoclave or by irradiation.) Media Fill is not required. Option not availabe lfor biologics.

- **Aseptic Processing** – where all materials, packaging and solution are **sterilized separately then assembled** aseptically to give final product. This requires media fill validation.

Sterility Assurance

- Sterility Test is limited – does not provide sufficient sterility assurance – PNSU < 14% (95% confidence)
- Media Fills are far more relevant PNSU < 0.1%(99% confidence)
- Only as good as critical parts & control of bio-burden:
  - Aseptic Operators Technique and Interventions
  - Sterilization Systems
  - HVAC Systems
  - Product filtration programs
  - Cleanroom / Facility / Pressure etc.
  - Cleaning and sanitation program
  - Movement of materials into Grade B and Grade A

Sterile Products and Risk

- With terminal sterilization, provided the bioburden is not too high, the final product will be sterile. The risk of having an unsterile product is very low. Very very few sterility based recall are from terminally sterilized product.

- With aseptic processing even if all components and solution are sterile **poor technique by an operator can introduce microbial contamination and make the product unsterile**.

- The more manual the process is, the higher the risk.

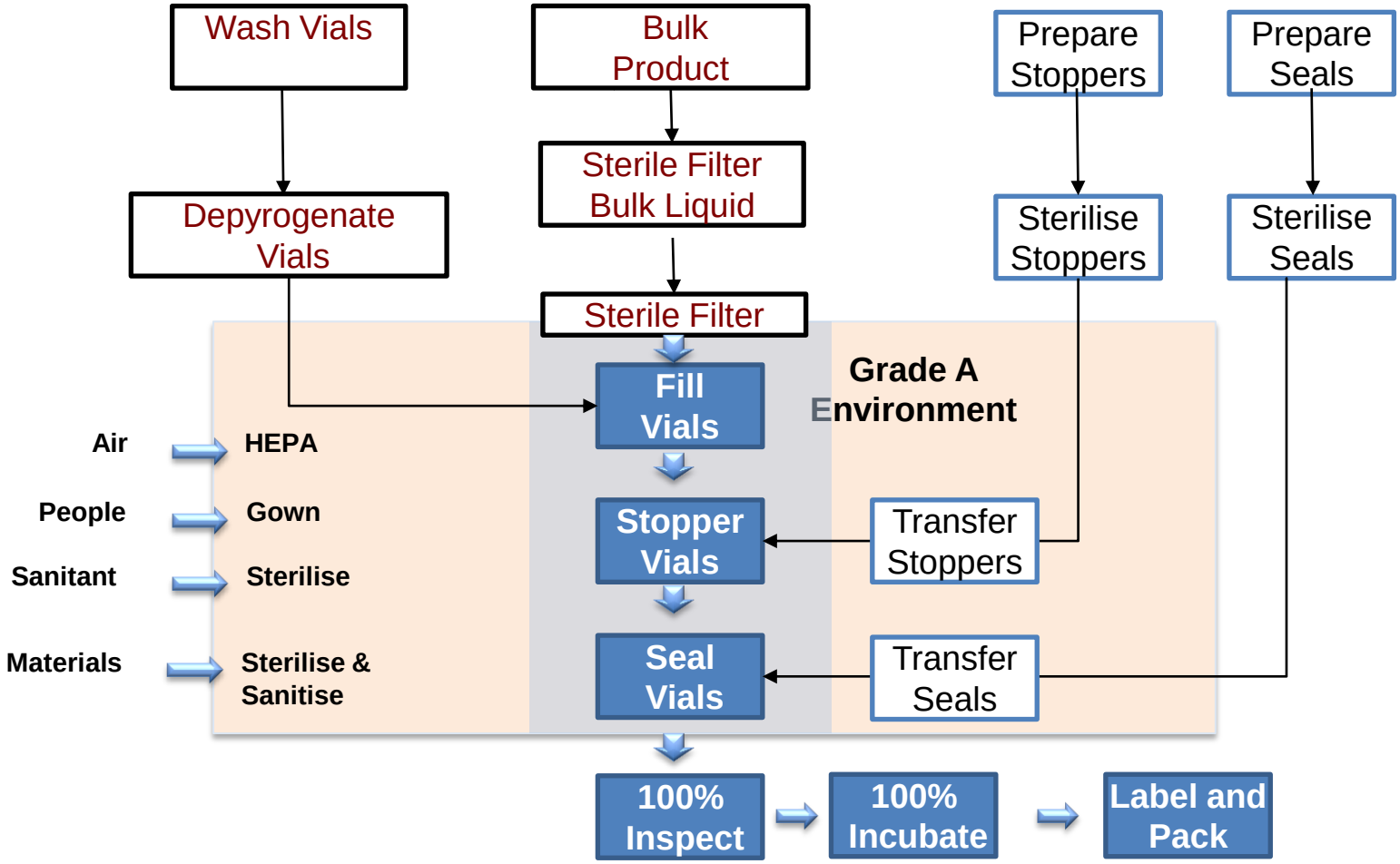
Minimizing contamination – Risk Rate the List!

Items	What we do to prevent microbial contamination
Vials	Sterilized and depyrogenated with dry heat oven or tunnel
Rubber Closures / Caps	Sterilized by autoclave
Chemicals	Tested to be sure microbial contamination is within specification.
Water for Injection	Held at high or low temperature and ozonated
Sundry items (scissors, scoops. Tweezers, etc)	Sterilized by autoclave / Hot Air Oven
Air Supply	Air is especially filtered to reduce chances of microbial problems. HEPA filters are tested regularly to verify efficiency.
Operators	Trained so they understand aseptic technique. Technique verified by media fill challenge
Garments	We use sterile garments to protect product
Production environment	We sample and test to verify absence of microbes
Bulk Tanks	Cleaned and sanitised – we test to show they are clean
Sterilising Filters	Are supplied sterile or sterilized in house

Industry Trend (Regulator Preference)

- Controlling contamination is always better than monitoring it
- Minimise / eliminate hand filling operations
- Expect minimal machine interventions – relies upon well tuned and reliable equipment.
- Separate operators from the exposed product
  - Semi closed Restricted Access Barrier Systems (RABS)
  - Fully closed RABS
- Isolator Technology

Aseptic Processing of Vials



Some Basic GMP Rules – cGMP Annex 1

- Low to no reliance on the sterility test
- Only sterilized or sanitized items in Grade B, then A
- Aseptic technique is critical – “worst case” challenged
- Aseptic operators must be qualified, re-qualified or dis-qualified
- EM programs must include set up as well as operation
- **Intervention = Risk.** Keep people remote from product
- Cannot be any air entrainment from B to A space
- Intensive monitoring program
- All incidents/events must be reviewed

Grade A
Critical Space and Critical Surfaces

Critical Space – Grade A / ISO 5

A critical space is one in which the sterilized drug product, containers, and closures are exposed to environmental conditions that must be designed to maintain product sterility.

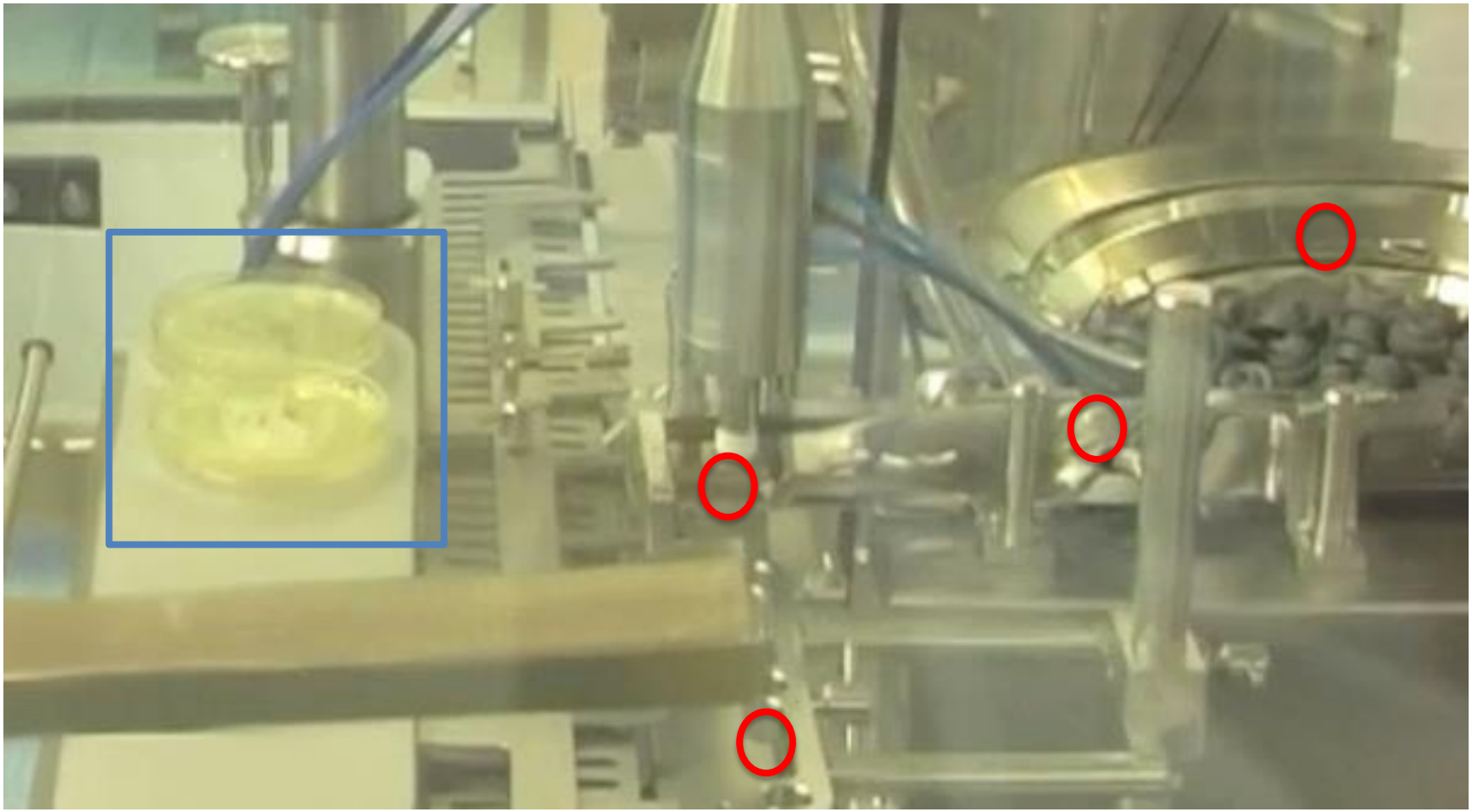
Critical Surfaces within Critical Space

Not all Grade A space is a critical surface.

Surfaces that may come into contact with or directly affect a sterilized product or its containers or closures.

Critical surfaces are rendered sterile prior to the start of the manufacturing operation, and sterility is maintained throughout processing. Generally monitored post processing.

Critical Space and Critical Surfaces



Air Visualization in Grade A Space

- Identify worst case locations for EM monitoring
- Look for turbulence in Grade A
- Look for entrainment B to A
- Must do in “at rest” state
- Must do in simulate “dynamic state” around interventions
- Must do whenever major change
- Should repeat periodically
- Must have a protocol and visualisation report prepared by QC/QA Microbiology
- Can use videos as training tools for aseptic operators

Key systems – people in red

People & Aseptic Processing

- People continuously shed microbes & particles into their surroundings; cleanroom garments do not contain all of the organisms present on human skin.
- People represent the main risk for non-sterile products
- The **presence** of contaminating microorganisms during aseptic interventions is largely unavoidable.
- The **transfer** of those organisms to the critical space is avoidable.
- Try to manage and control the level of unplanned interventions.

Personnel: Aseptic Personnel Qualification Program

- Demonstrate an understanding of applicable Standard Operating Procedures (SOPs)
- Demonstrate an understanding of Basic Microbiology
- Demonstrate an understanding of Aseptic Practice Theory and Cleanroom behavior
- Demonstrate gowning proficiency by actually completing three consecutively successful gownings.
- Successfully complete a “Media Transfer Evaluation” within a Grade A hood in a laboratory environment demonstrating successful aseptic technique simulating interventions.
- Successfully participate in a process simulation (media fills) annually – covering interventions

Other Personnel Management Rules

- Cannot be in Grade A until fully qualified – assistant in Grade B
- Frequent glove/gown surveillance – if failing must have re-training and re-qualification. Maintain a table of results
- Dis-qualified if cannot meet standards
- Any positive on Grade A gloves is a problem – must be investigated

Media Fill Validation

- Evaluates the entire process
- Must occur every 6 months per process line per shift
- Must include all aseptic operators over time eg. annually
- Must include “ancillary” staff who have to enter the room
- Must be “worst case” challenge to the process:
  - Routine and non-routine interventions by each operator
  - Different container – closure combinations
  - Maximum # personnel in the room
  - Changeovers and sterile hold times for equipment
  - 100% inspection process
- Run size: 5000 or maximum # processed on lien for the container closure combination. Pass = NIL positives

In an ideal (risk free) world
every potential risk would be covered in the media fill

- Every sterile bulk hold period would be simulated for the maximum hold period
- Aerobic and anaerobic media would be used
- Simulation of the maximum permitted bulk hold time and maximum filling time
- Every possible intervention, stoppage, process, procedure or worst case situation would be simulated
- Every possible container/closure combination would be tested
- Every aseptic operator would perform every intervention 3 times
- > 10,000 container per run and would be zero positives

Risk & Aseptic Processing Tasks

Task	Ease of Validation	Reliance on Personnel	Associated Risk
Sterilization	Easy	Low	Low
Room Design	N/A	N/A	Moderate
Monitoring	Moderate	Variable	High
Sanitisation	Difficult	High	High
Gowning	Difficult	High	High
Material Transfer	Difficult	Very High	Very High
Aseptic Technique	Difficult	Very High	Very High
Aseptic Assembly	Difficult	Very High	Very High

Process Interventions

- **Principle**:
  - **Avoid interventions,**
  - **Where they are unavoidable, minimize their impact.**

- **Routine interventions** are activities that are inherent parts of the aseptic process and integral parts of every batch.

- **Non-routine interventions** are activities that are predominantly corrective and may not be a part of every batch.

Risk Rating of Interventions

Risk Rating	Intervention Activity	Potential Contamination Risk	Frequency of inclusion in media fill	Glove monitoring post intervention
5	Critical Surface or aseptic connection	Very High	Every Fill	Yes
4	Proximity to an open container	High	Every Fill	Yes
3	Remote to an open container/closure	Medium	Once per year	No
2	Post Capping	Low	Once per year	No
1	Grade B activity	Very Low	Once per 2 years	No

Routine Interventions

Routine interventions are interventions that are normal parts of aseptic processing. These may be:

- Aseptic assembly of equipment before use (stopper bowl, cap bowl etc.
- Adjustment of the machine tracks
- Initial product connections (i.e. to filler or to filter)
- Siliconing of the vial turntable
- Fill weight checks
- Bubble point testing of filters
- Component additions (vials, stoppers, caps)
- Environmental monitoring
- Any other intervention that is part of the normal process
- Stoppages due to meal or rest breaks

Non-Routine Interventions

Non-routine interventions are any interventions that are corrective and are or **should be** uncommon. Examples are:

- In process adjustment of the machine tracks
- Removing defective seals on containers
- Removing vials from the line that have jammed the machine
- Removing vials from the line that have fallen over
- Product filter change (initial bubble point failure ?)
- Replacement of filling needle or hose
- Product spillage or leakage
- Poorly fitting stoppers that require more manual manipulation
- Any other problem that requires manual correction

Grade A Intervention Rules

- Make sure the machine is stopped first
- ALWAYS sanitise hands thoroughly before going into Grade A space
- Keep as much of your body as possible out of the cabinet
- NEVER lean over to top of an open vial, stopper/cap bowl or the filling needle.
- Use sterile forceps to retrieve or remove upturned vials
- Do not intervene to remove stoppers, vials, caps that are not interfering with processing – clean up at the end
- Sample gloves post intervention
- Practice good aseptic technique EVERY TIME!

Process Validation - Media Fills

- The Media Fill Trial is a simulation of the filtration and aseptic filling process, which substitutes a microbiological growth medium for a sterile product.
- The Media Fill Trial provides to evaluate aseptic processing operations that may affect the sterility of the final product, and the performance of aseptic filling personnel under operational conditions.

Pre-requisites to Process Validations

- Critical area are qualified and HVAC HEPA filters certified
- Environmental monitoring procedures are qualified
- Environmental monitoring media is approved for use
- Equipment and component sterilisations steps are validated
- Sterile filtration of bulk products is validated
- Media used in simulations is qualified
- Staff involved in the media fill are qualified in aseptic gowning
- Staff entering the filling area are trained in aseptic technique.
- Media fill inspectors are trained to detect turbidity

Elements of Aseptic Process Validation
(FDA Guidance – 2004)

- Media Fill Conditions / worst case situation / What are the risk factors ?
- Frequency and Number of Runs
- Duration of Run
- Size of Run
- Line Speed
- Environmental Conditions
- Media
- Incubation and Examination of Media-Filled Units
- Interpretation of Results

Study Design

- Objective:
  - A media fill program should accurately assesses the state of process control.
- Approach:
  - Incorporate the contamination **risk factors** that occur on a production line
  - Closely simulate aseptic manufacturing operations incorporating worst-case activities and conditions that provide a **challenge** to aseptic operations.

When are Media Trials Performed?

Frequency and Requirements for Challenges

- New Production Lines
- Changes to Existing Production Lines
- Routine Re-validation of Aseptic Filling Lines
- Facility Shutdowns and Recommissioning

Q. Identify at least three (3) changes that would warrant re-validation.

Number of Runs

- Principle:
  - Minimum: **three (3)** to qualify the line initially
  - Maximum: Enough to ensure that results are consistent and meaningful.
- Routine re-validation: each processing line every 6 months.
- **All** personnel who are authorized to enter the aseptic processing room during manufacturing should participate at least once a year.
- Participation should be consistent with the nature of each operator's duties during routine production.

Duration of a Media Fill?

- The duration of the run should:
  - Simulate the expected maximum time for routine manufacture
  - Include all production shifts.
  - Be dictated by the time needed to fill the required number of units
  - Ensure that the necessary number of units and activities are included.
  - The validated maximum run tome should be included in batch records

How Many Units (Containers) should be filled

- The number of units should be large enough to yield a high probability of detecting low incidences of contamination.
- A minimum of 5000 containers, or the normal batch size, whichever is the least, should be included in any one simulation
- Ideally the media fill should be equivalent to the runs size – this is controversial and no universal rule.
- Must have enough units to run the maximum allowed fill time

** At least 4,750 (5000) units are needed to detect, with 99% probability (confidence), a contamination rate of one in one thousand.

Some Binominal Statistics

N = log (1 - c) / Log (1 – p)

N is the number of units examined

c is the confidence level required (95% or 99%)

p is the Lot tolerance defective level (p <0.001)

Simulation Conditions - Line Speed

- The media fill program should adequately address the range of line speeds employed during production.
- Each media fill run should evaluate a single line speed, and the speed chosen should be justified.
- Exercise:
  - Identify the situations that would be best evaluated by a high line speed and a slow line speed.

Simulation Conditions - Environmental Conditions

- Principle:
  - Media fills should be adequately representative of the conditions under which actual manufacturing operations are conducted.
- To the extent standard operating procedures permit stressful conditions it is important that media fills include challenges to support the validity of these studies.
- Exercise:
  - List stressful conditions that you include in an aseptic process validation study.

Simulation Conditions - Media

- **Principle**:
  - Optimize detection of any microbiological contamination.
- Soybean casein digest medium (SCDM), should be used as it promotes growth of gram-.positive and gram-negative bacteria, and yeast and mold
- Consider inclusion of anaerobic growth media (e.g., fluid thioglycollate medium) if there is a relevant risk factor.
- Need to consider production related isolates (use own isolates).
- Media must contact all of each unit (container and closure)

Incubation of Media-Filled Units

- Principle:
  - Media units should be incubated under conditions adequate to detect microorganisms that might otherwise be difficult to culture.
- Incubation temperature should be:
  - suitable for recovery of bioburden and environmental isolates and should at no time be outside the range of 20-35^{o}C.
  - maintained within +2.5^{o}C of the target temperature.
- Incubation time should not be less than 14 days.
- If two temperatures are used for the incubation of the media filled units, the units should be incubated for at least 7 days at each temperature (starting with the lower temperature).**

** controversial (PICs recommends 2 temperatures)

Examination of Media-Filled Units

- **Each** media-filled unit should be examined for contamination by personnel with appropriate education, training, and experience
- QC Microbiology oversight throughout any such examination.
- All suspect units identified during the examination should be brought to the immediate attention of the QC microbiologist.
- Use clear containers (with otherwise identical physical properties) for amber or other opaque containers.
- When a firm performs a final product inspection of units immediately following the media fill run, all integral units should proceed to incubation. (Non-integral units should be separately incubated.)

Acceptance Criteria and Responses

- The target is zero positives.
- **Any positive unit indicates a potential sterility assurance problem, regardless of run size.**
- All positive units should be identified (speciated) and should result in a thorough, documented investigation by microbiology and production

Acceptance Criteria and Responses

- When more than 5000 units are filled, caution should be used when deciding to increase the allowable number of positives. {Note: more than 1–2 positives, regardless of the size of the simulation, may be difficult to justify, and thus accept on quality grounds, without corrective action}.

- If the positive units are indicative of an unacceptable practice (e.g., an incorrect or inappropriate type of intervention) it should be corrected after a risk assessment.

Additional media fills may be required in response to the following:

- Where routine shutdown of a dispensing line has occurred to perform maintenance or engineering project activities.
- Non-routine maintenance or engineering project activities are performed that require significant modification of the Dispensing Line or HVAC system in a dispensing line. The requirements for a media fill will be determined via project related risk assessments.
- A dispensing line is decommissioned permanently or for a significant period of time (greater than 6 months).

100% Inspection of Filled Units

- GMPs require 100% visual inspection of filled units
- This can be by automated (camera), semi automated (mechanical presentation to a viewing station) or manual inspection.
  - All the above have advantages and disadvantages
- Must have an SOP and a Record of inspection
- Where people are used to inspect they must:
  - Be trained in inspection
  - Be tested to be able to accurately select a range of different defects
  - Be rotated out so they do not get tired
  - Be eye tested annually

100% Inspection of Filled Units

- Defects should be classified in relation to patient risk
- Defects should have individual attribute limits plus there should be an overall limit of permitted defects for the process
- Industry now have also adopted an independent inspection using sampling and AQL limits

- **Can you think of some defect classes ?**

Some Questions to Discuss

General Sterile Products Quiz ?

- What is lower risk terminal sterilization or aseptic processing ?
- Why would companies choose one over the other ?
- Which is better Quality Assurance – a media fill pass or a sterility test pass ?

Environment Quiz ?

- Should airflow pattern studies be videotaped?
- What are the recommended particulate and microbial alert and action levels for aseptic processing areas?
- Are environmental monitoring alert and action levels considered specifications
- What is the relationship between environmental monitoring and batch release?
- Is continuous viable environmental monitoring required in Grade A (Class 100) areas?

Environmental Monitoring Quiz ?

- When and where should air monitoring be conducted for **non-viable particulates** (NVPs)?
- When and where should air monitoring be conducted in aseptic Cleanroom for **viable particulates** (VPs)?
- Should viable monitoring be expected on sterilized product contact surfaces?
- What microbial identification strategy is expected Grade A environmental monitoring samples? Why ?
- Should personnel be monitored when they conduct an interventions ? Why ?

Personnel Quiz ?

- Explain why personnel behavior is critical in aseptic processing ?
- What is the difference between a Grade B and a Grade A operator ?
- Under what circumstances could a Grade A operator be “dis-qualified” ?
- Once an aseptic operator passes a media fill they should not have to repeat the interventions program. Discuss.

Process Validation (Media Simulation) Quiz ?

Should non-integral media fill units be incubated?

What type of interventions are required for process simulations, and with what frequency?

How many units should be processed in a media fill ? Why ?

What should I do next if a find one positive media fill unit in a run of 5000 ?