Centre for Biopharmaceutical Excellence

Sterile Manufacturing of Vaccines: What Regulators and Inspectors Look For.

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 sterile products
- Aseptic Processing GMP Expectations
- Fundamentals of Environmental Monitoring
- Sterilisation 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 available for biologics/vaccines.

- **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)

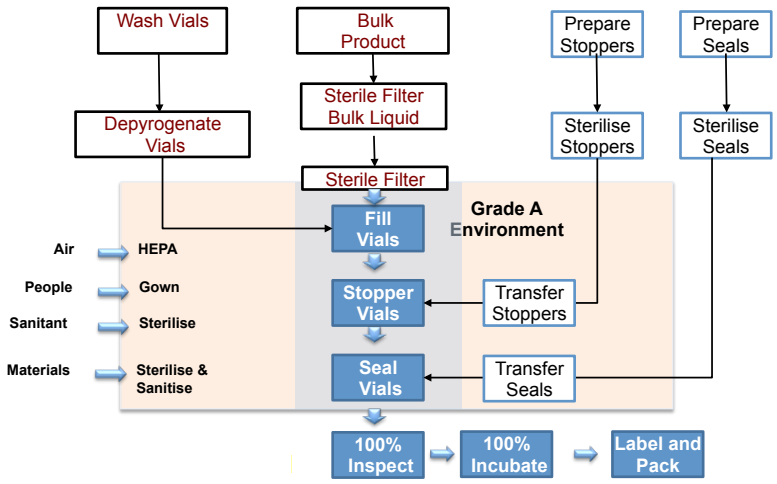
- Media Fills are far more relevant PNSU < 0.1%(99% confidence)

- 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.

Aseptic Processing of Vials



Minimizing Contamination – Risk Rating
Low, Medium, High

Items	What we do to prevent microbial contamination
Vials	Sterilized and depyrogenated with dry heat oven or tunnel
Closures / Caps	Sterilized by autoclave
Disinfection	Disinfectants are qualified and sterilised before use
Water for Injection	Held at high or low temperature and ozonated
Sundry items (scissors, scoops. Tweezers, etc)	Sterilized by autoclave / Hot Air Oven. Handling becomes the major risk
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. Direct link between proximity to open product and risk of contamination
Garments	We use sterile garments to protect product
Production environment	We sample and test to verify absence of microbes
Material Movement	Movement into Grade B and A represent high potential risk
Sterilising Filters	Are supplied sterile or sterilized in house

FDA View on Minimising Aseptic Processing Risk

https://www.youtube.com/watch?v=z2u3_tH7nAE

- 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

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.

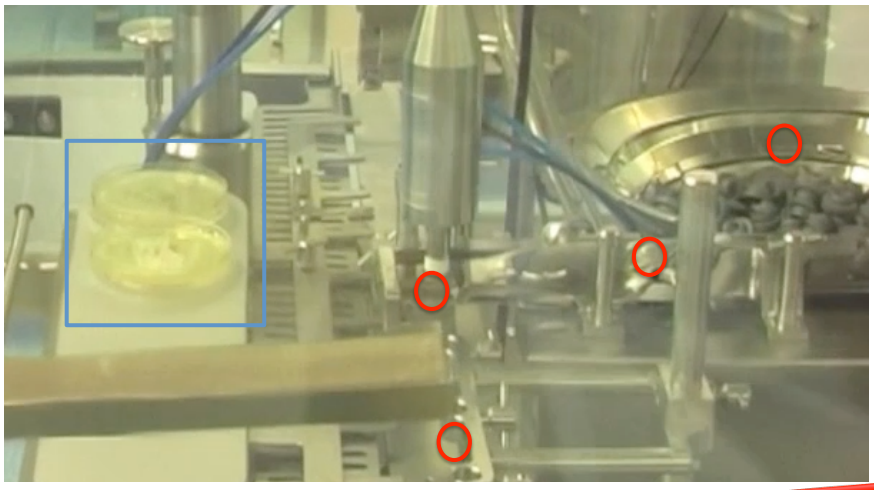
Critical Surfaces within Critical Space

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 https://www.youtube.com/watch?v=6JfN6RezDkA

People & Aseptic Processing

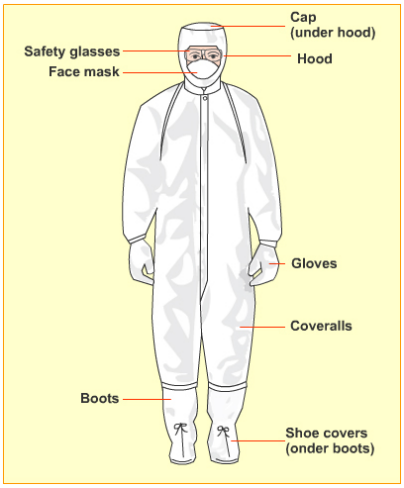
- People continuously shed microbes & particles into their surroundings; cleanroom garments do not contain all of the organisms present on human skin.
- People represent the main risk for non-sterile products
- The **presence** of contaminating microorganisms during aseptic interventions is largely unavoidable.
- 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 Process 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

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

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

Aseptic Process – Risk Based Interventions

Risk Rating	Intervention Activity	Potential Contamination Risk	Frequency of inclusion in Media Fill	Glove monitoring required post intervention
5	Critical surface** in Grade A	Very High	Every Fill	Yes
4	Proximity to an open container	High	Every Fill	Yes
3	Remote to open container	Medium	Every Fill	No
2	Inside Outer Grade A area	Low	Once per year	No
1	Outer Grade A and Grade B Area Activity	Very Low	Once per two years	No

No.	Intervention Description	Type of Intervention	Risk Rating	Number of repetitions	Required for Grade A Operator Qualification
1	Interchange the position of the operators	Planned	3	Throughout	-
2	Adjust fill weights	Planned	3	Once	-
3	Slow fill to expose session to maximum time allowed-6 - 8 hours	Planned	3	Throughout the session	-
4	Maximum number of staff in the room during the fill session 5 operators	Planned	3	Throughout the session	-
5	Remove fallen/jammed vial(s)on rotation table	Unplanned	4	Grade A ops once	Yes
6	Clear jammed stopper in track	Unplanned	5	Grade A ops once	Yes
7	Clear jammed cap in track cap	Unplanned	2	Grade A ops once	-

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!

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

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.

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.

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°C.
  - maintained within +2.5°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 ?**

Flash Quiz

	Aseptic Processing	Your Selection
1	(a) Terminal sterilisation is higher risk than aseptic processing (b) If the media fill fails but the sterility test passes the process line is OK (c) Only 1000 units need to be processed in a media fill (d) Only the best aseptic operators should conduct media fills	
2	a) Interventions should be risk rated b) All interventions have the same level of risk c) The maximum process time should be simulated in media fills d) 100% inspection defects should be classified into type	
3	If 5000 units are processed and they all pass the we can be 99% sure the defect level is < 0.1%	TRUE/FALSE
4	Once an aseptic operator passes a media fill they should not have to repeat the interventions program.	TRUE/FALSE

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Microbiology Controls
Environmental Monitoring Programs

Module Topics

- Fundamental EM Program – what to monitor
- Frequency, Location and Methods
- Monitoring Water Systems
- Managing an EM program for Sterile Cleanrooms

Some Important References

- PDA Technical Report #13 Fundamentals of an Environmental Monitoring Program
- USP <1116> Microbiological Evaluation of Cleanrooms
- FDA Guidance – Aseptic Processing
- ISO 14644 Series - Cleanrooms and associated controlled environments

Why do we monitor ?

- **Particulates:** Verify that the HVAC systems is functioning correctly and rooms are meeting specifications. Particles are associated with physical contamination and indirectly micro-biological contamination.

- **Microbiological:** The purpose of viable environmental monitoring is to:
  - verify the integrity of the cleanroom air and HVAC systems
  - monitor the effectiveness of surface C&S programs
  - monitor operator performance via personnel monitoring.
  - Monitor aseptic process integrity in Grades A and B

What should be monitored ?

Physical

- Particles at rest and in operation in Grade A and B
- Temperature
- Relative Humidity
- Room Pressures
- Room Air Change Rates
- HEPA Integrity

Biological

- Bacteria
- Yeast and molds

Pharmaceutical Critical Services

- Water system and in some cases steam
- HVAC
- Pharmaceutical gases (compressed air , nitrogen)

What Methods (Biological) ?

Each method has advantages and limitations

Suitable **combination** of:

- **Settle plates** – passive limited space but extended time coverage
- **Contact (RODAC) Plates** – small flat surface areas
- **Surface Swabs** – larger inaccessible areas but harder to recover
- **Active Air Sampler** – better detection but limited time duration
- **Gloves and garments monitoring** – hit or miss.

What Sample Locations to Choose ?

- Defined for particulates generally in ISO14644 and cGMP Annex 1
- **Risk based decisions** for monitoring locations
- Microbiological sampling driven by the **purpose of sampling**
- Considerations:
  - Proximity to the product
  - Product contact equipment surfaces
  - Whether testing for cleaning verification or product purity

Example Locations – Sterile Suites

Grade A Space

- Generally only at or above working height
- Near open filled containers (air sample)
- Critical surfaces (post filling swab only)
- Obstacles that may create turbulence (air)
- Curtains and machine doors
- Beneath equipment / floor ?
- Post changeover of lines

Grade B Space

- Proximal to air returns
- Door handles (swabs)
- Obstacles that may create turbulence (air)
- Trolleys
- HMI Consoles
- Floor / Walls/Windows
- Pass throughs
- Adjustment tools

Higher Risk Locations in Grade A

- near open filled containers (air sample)
- proximal to air return (air sample)
- screens, curtains and door handles (swabs)
- critical surfaces (post filling swab only)
- gloved hands (contact plate)
- obstacles that may create turbulence (air)

Example Locations – Grades C and D

- Post processing product contact equipment cleaning surfaces
- Hardest to clean locations for direct product contact surfaces
- Rooms with open processing – more frequent
- Rooms with “contained” processing – lesser frequency
- Rooms with closed processing – less frequency
- Non-processing rooms – infrequent
- Air locks
- Drains, washbays ?

How Frequently to Monitor ?

- Is a risk based decision which depends on:
  - the dose form being processed (aseptic / terminal)
  - whether the processing is **"closed", "contained"** or **"open"**
- Sterile products / aseptic processing have defined requirements for Grade A, somewhat for Grade B.
- Grade A and B expect some frequent/continuous coverage – settle plate exposure enables this
- Need enough samples to conduct trending over a year
- Some fixed locations and some rotational

WHO Recommended Viables Monitoring Frequencies

Table 5. Microorganism *in-operation (dynamic)* routine monitoring frequencies

Classification	Volumetric[2]	Settle plate[2]	Contact plate	Glove print
Grade A (filling operations)[1]	Once per shift	Once per shift	Once per shift	Once per shift
Grade B	Daily	Daily	Daily	Daily
Grade C	Weekly	Weekly	Weekly	N/A
Grade D	Monthly	Monthly	N/A	N/A
UDAF in B	Once per shift	Once per shift	Once per shift	Once per shift
UDAF in C	Weekly	Weekly	Weekly	Weekly
UDAF in D	Monthly	Monthly	Monthly	N/A

(2) The practice of air sampling at the start, middle, and end of filling operations provides better environmental monitoring and facilitates investigations related to filling batch release. This approach should be part of a general environmental monitoring strategy based on risk analysis and considering the types of activities performed.

The Paperwork

- Sample Location
- Date sample taken (length of time for settle plates)
- Batch number and expiry of the media
- Operator(s) who took the samples
- State of the room (at rest or in operation and activity)
- Incubation conditions
- Operator reading the plates and date read
- Number of cfu per sample – separate for Yeast / Mold
- Any identification
- Signature of person reviewing the results

Physical Monitoring

Pressure Differentials

- Generally continuous by a validated EMS or
- Magnehelic gauges outside each processing room read twice daily
- Generally > 15Pa (sterile) and > 10Pa (non- sterile) differentials
- Verify air flow directions between rooms “at rest”

Temperature / RH %

- Either EMS system or in- room physical monitors
- Record Max and Min per day

Filter Integrity

- Annual clean and test for % penetration
- Velocity not usually measured for non-sterile rooms, except for validation purposes / air change rate calculations.

Particulate Monitoring (WHO)

Table 2. Maximum permitted airborne particulate concentration per air grade[2]

Grade	At rest		In operation	
	Max. permitted particles / m^3		Max. permitted particles / m^3	
	≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Table 3. Monitoring frequencies for *in operation* routine particulate sampling

Classification	In operation (dynamic) routine particulate sampling
Grade A (filling operation)	For the full duration of operation
Grade B	Daily[1]
Grade C	Weekly
Grade D	Not required
UDAF work stations in B	Daily [(1)]
UDAF work stations in C	Weekly
UDAF work stations in D	Monthly
UDAF in UNC areas	Routine re-qualification of UDAF is sufficient

[(1)] Working days. Monitoring can be omitted on e.g., weekends if no production activities are taking place.

Developing and Managing a Cleanroom Microbiological Monitoring Program for Sterile Products

Who Should Monitor ?

Oversight by QC Microbiology

QC Led Team

- Independent of Production
- Policeman approach
- Not efficient utilisation of resources

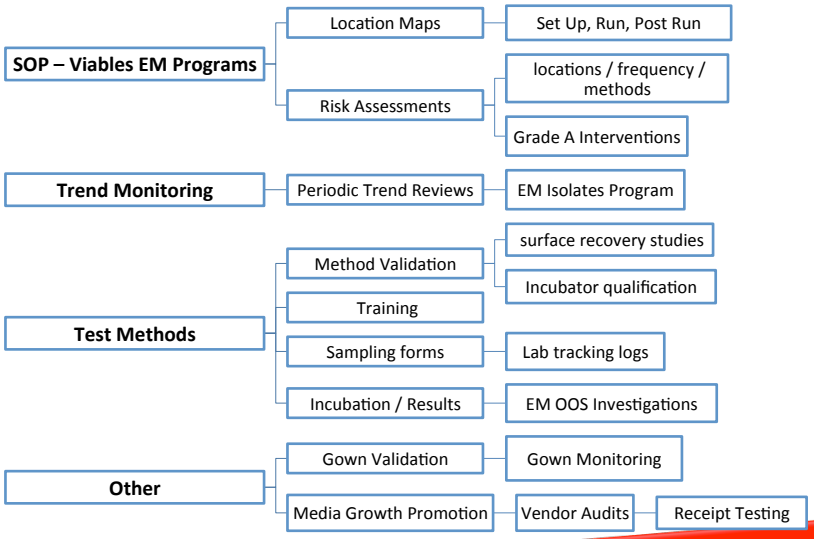
Production Led Team

- Pragmatic approach
- In-process control
- Must have strong QC oversight
  - Training of operators
  - QC surveillance program
  - Random audits by QC

Establishing a Viables EM Program for Grade A and B

1. Engage Microbiologist and Aseptic Operators
2. Study the fill line, process, critical space and Grade B Rooms
3. Study personnel and materials movement
4. Conduct air visualisation studies “at rest” then in “simulated operation”
5. Characterisation study for extended period (if possible) grid rooms
6. Risk assess worst case locations:
   - Critical space and critical surfaces
   - Areas with high activity or personnel frequently in proximity
   - Areas with high personnel traffic or areas frequently touched
   - Areas difficult to sanitise effectively
7. 3 times OQ at rest after C&S program
8. 3 times PQ in operation after C&S program
9. SOP - Fixed and rotational locations in 1st 12 months – review 3 monthly then update after review.

Core EM Documentation



Physical Environment and Airflow Patterns

https://www.youtube.com/watch?v=6EfOLDaRu2o

- Airflow patterns are studied (visualization studies) to:
  - Look for lack of turbulence and no entrainment across Grade B to A interfaces;
  - Identify worst case locations for EM sampling sites
- Must do under “at rest” and simulated “in operation” modes
- Airflow patterns are established during qualification and re-validation studies to ensure the validated conditions have not changed;
  - Grade B to A interfaces
  - Movement through pass through cabinets (PTCs)
- The patterns should be documented so changes can be detected. Require a protocol and report + raw data (video)

GMP Rules for Grade A and B Air Monitoring

- **Non-viable Particles (NVPs)**
  - Grade A must be continuous monitoring during set up and operation
  - Grade B continuous not mandatory, but preferred
  - Must have an SOP for excursion and line clearance
  - There is an “association” between NVPs and microbes
- **Viable Particles (VPs)**
  - Variety of techniques – all have reasons and challenges
    - Settle plates (passive), contact plates, active air, surface swabs
  - Must be continuous monitoring in Grade A and Grade B
  - Must monitor operator gloves, post intervention and on exit of room and gowns periodically
  - Must have alert and action response program

Site Selection Considerations

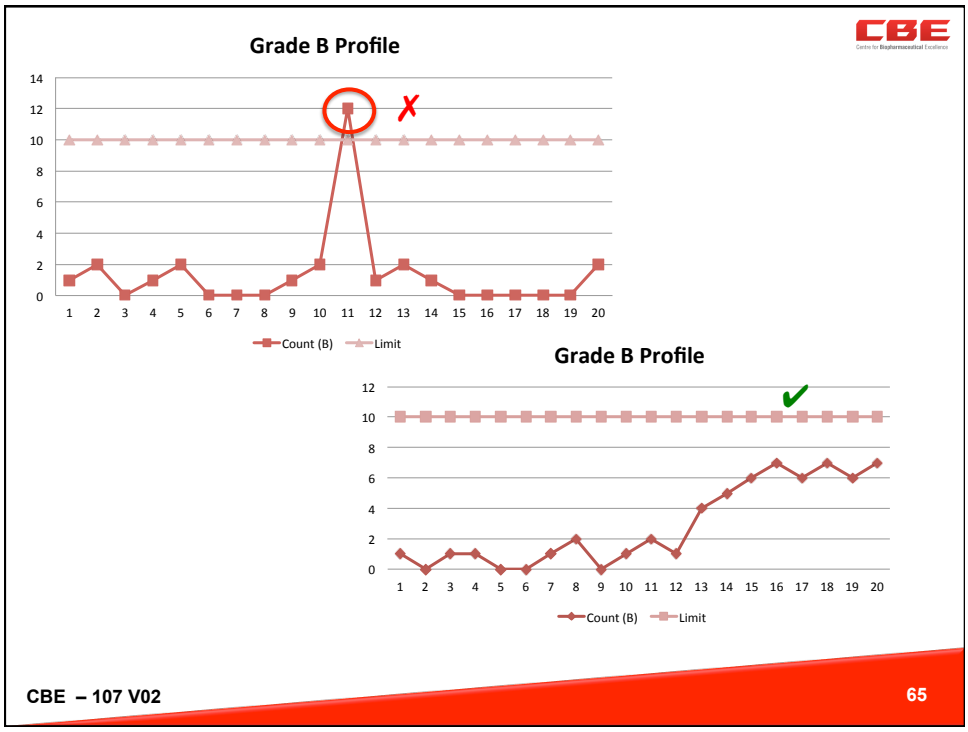
- Can use a combination of grid mapping by room and risk assessment by location in room
  - Sites or process steps where contamination may adversely effect product
  - Sites likely to accumulate microbial load during processing or use
  - Potential “dead spots” in room
  - Sites most difficult to clean or sanitise
  - Means of microbial dispersion in the room environment via:
    - People, equipment, processes, materials and air flows
- Must also consider risk to product associated with the sampling itself
- Must be able to remove any media residue from surfaces
- Must not interfere with operators during processing

Interpreting Viable Industry Limits
(Grade A and B Space)

	Active Air cfu per m^3	Passive Air (Settle – 4 hr)	Surface (Rodac/Swab)	Personal (Glove 5 finger)	Personal (Gown)
EU/PICs/Who Annex 1	A < 1 B 10	A < 1 B 5	A < 1 B 5	A < 1 B 5	Not specified
US FDA Class 100 Class 10,000	1 10	1 5	Not specified	Not specified	Not specified
USP <1116> (incident rate)	ISO 5 <1% ISO 7 <5%	Same incident rate as active air	Same incident rate as active air	Same incident rate as active air	Same incident rate as active air
Japan Aseptic Guide (JPXV1)	A < 1 B 10	A < 1 B 5	A < 1 B 5	A < 1 B 5	Not specified

Interpreting Viable Industry Limits
(Grade C and D Space)

	Active Air cfu per m^3	Passive Air (Settle – 4 hr)	Surface (Rodac/Swab)	Personal (Glove 5 finger)	Personal (Gown)
EU/PICs/Who Annex 1	C 100 D 200	C 50 D 100	C 25 D 50	Not specified	Not specified
US FDA Class 100,000	100	50	Not specified	Not specified	Not specified
USP <1116> (incident rate)	ISO 8 <10%	Same incident rate as active air	Same incident rate as active air	Same incident rate as active air	Same incident rate as active air
Japan Aseptic Guide (JPXV1)	C 100 D 200	C 50 D 100	C 25 D 50	Not specified	Not specified



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Minefields in Limit Interpretation

- Numbers matter but trend changes matter as much
- PICs says “on average” but single excursions matter
- Based on total counts but species matter e.g yeast/mold, pseudomads …..
- Limits have caveats regarding methods / conditions
- Grades B, C and D limits are generally excessive in a well controlled cleanroom
- These are regulatory or “action” limits – companies expected to develop “alert” levels
- USP <1116> proposes incident rates instead of numbers – basis is trend monitoring.
- Significance is related to the proximity to open product

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Analysis of Microbiological Data (Modular or Product Specific)

- Cleanroom Environmental Monitoring (Modular)
- Bioburden (can be both Product and Modular)
- Water Systems (Modular)
- Steam and Compressed Air Systems (Modular)

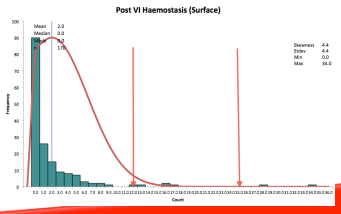
- Cannot assume “normal” distribution of data
- Most values tend to be “0” – hard to mathematically treat
- Must use other techniques
  - Log e or 10 conversion approach
  - Rank Percentile approach

Analysis of Microbiological Data (Objective)

- Want to verify or re-establish **alert and action limits** based on historical trends.

- Science based approach:
  - Convert to Log10 or Log e – this tends to normalise data = problem of zeros.
  - **Rank in order and cut off at say 95% (action) and 90% (alert)****
  - Use cumulative frequency approach

** minimal mathematics needed



Using Recovery / Contamination and Incident Rates (Refer to USP – 1116)

Recovery (Contamination) Rate: Number of samples with positive results expressed as a percentage of total samples

Incident Rate: Number of samples with results above the alert /action limits expressed as a percentage of total samples

Manufacturing Facility	Grade C		Grade D	
	Air	Surface	Air	Surface
Number of Samples	237	589	150	409
Number of Samples with growth	95	354	126	253
Recovery Rate	40.1%	60.1%	84%	61.9%
USP <1116> recommendations for recovery	< 5%	< 5%	< 10%	< 10%
Limits (Alert / Action)	50/100	12/25	100/200	25/50
Number of OOL Alert Incidents	2	33	0	12
Number of OOL Action Incident	1	9	4	18
Incident Rate: Alert	0.8%	5.6%	0 %	2.9%
Incident Rate: Action	0.4%	1.5%	2.7%	4.4%
Incident Rate: Total	1.2%	7.1%	2.7%	7.3%

Example of Good EM Annual Review

Grade	Type of Monitoring	Filling Room # 1	Filling Room # 1 Vial Storage	Filling Room # 2
A	Active Air	561	187	19
	Passive Air	561	187	19
	Surface	1587	0	56
	Total EM Samples	2659	374	94
	Number Positives	0	0	0
B	Active Air	561		38
	Passive Air	0		19
	Surface	2171		220
	Total EM Samples	2732		277
	Number Positives	0		0

Alert and Action Limits (based on)

- Risk assessment for new production lines
- Historical trends for established process lines
- Compendial and regulatory guidelines - sterile
  - USP General Information Chapter<1116> Microbiological Evaluation of Clean Rooms and Other Controlled Environments
  - EU-GMP Annex I Manufacture of Sterile Medicinal Products
- All monitoring results should be reviewed regularly to detect trends and to confirm the effectiveness of the cleaning and sanitation program
- Generally QA/QC review: monthly, quarterly and as part of annual review program.

Example Setting Action and Alert Levels Using Ranking

cfu count	Rank	Percent	
59	1	100.0%	
33	2	97.9%	Action @ 95%
22	3	95.9%	
18	4	93.8%	Alert @ 90%
18	5	91.8%	
18	6	89.7%	
17	7	87.7%	
16	8	85.7%	
14	9	83.6%	
12	10	81.6%	
11	11	79.5%	
11	12	77.5%	
10	13	75.5%	
10	14	73.4%	
10	15	71.4%	
etc ….	etc …..		

Range of approaches used but need to set alert / action limits scientifically;

Ranking cut off is only one approach;

Must have sufficient data available;

Action Limit ≤ Regulatory limit;

Exceeding alert limit is not grounds for corrective action;

Rank Percentile Approach to Establishing Alert and Action Limits.

Data1		Point	Data1	Rank	Percent	
30		53	320	1	100.0%	
5		1	30	2	97.2%	99th Percentile
25		49	30	2	97.2%	
15		3	25	4	94.4%	95th Percentile
5		54	25	4	94.4%	
5		10	20	6	93.0%	
15		4	15	7	87.5%	90th Percentile
15		7	15	7	87.5%	
5		8	15	7	87.5%	
20		12	15	7	87.5%	
5		2	5	11	77.7%	
15		5	5	11	77.7%	
5		6	5	11	77.7%	
0		9	5	11	77.7%	
0		11	5	11	77.7%	
0		13	5	11	77.7%	
0		18	5	11	77.7%	
5		14	0	18	0.0%	
0		15	0	18	0.0%	
0		16	0	18	0.0%	
0		17	0	18	0.0%	
0		19	0	18	0.0%	
0		20	0	18	0.0%	

Action Limit

Alert Limit

Example Alert and Action Responses

If ……	Then ….. also refer to SOP xxxx
Any result exceeds the alert limit (or there is a trend)	• Identify the organism to genus level • Inspect the cleaning record for the equipment to verify it was properly cleaned and sanitized • Notify the QC Manager of the result • Initiate Alert Report (F xxxx) to notify the QA Manager and Production Manager
Any result exceeds the action limit	• Identify the organism to species level • Inspect the cleaning record for the equipment to verify it was properly cleaned and sanitized • Review the testing trends for all equipment used in non-sterile production • Notify the QA Manager of the result – determine whether a product risk assessment is warranted, or not. • Test the product for the absence of the identified organism • Initiate Deviation Report (F xxx) to notify the QA Manager and Production Manager

EM Excursions and Product Quality

- *"Alert and action limits do not define product attributes such as sterility and therefore should not be considered as product specifications or extensions of the product specification."*
- *"Rather they are intended to indicate changes so that corrective action may be taken before product quality is adversely affected."*
- *"Investigations are expected for action level excursions or adverse trends." (Using a written investigation plan)*

PDA TR 13 EM Fundamentals 2014

The type of bug matters !

- Mold is a problem in any facility – hard to remove.
  - Set mold limits lower than for total bacterial count limits
- Pseudomonas sp. in water systems and liquids and creams areas
- Known objectionable organisms / pathogens

Should occasionally "speciate" the organisms detected and always when there are unusually high numbers or during an investigation.

Recommended Incubation Methods

- Minimum of two temperatures to detect both bacteria and fungi.
- 3 to 5 days of incubation at 20 to 25oC followed by incubation 30 to 35oC for an additional 2-3 days
- The method chosen should be carefully validated and standardized.
- Option for Separate Incubation Conditions:
  - Y & M: 20°C - 25°C for 5 days (SAB or TSA plates) and
  - Bacteria: 30°C - 35°C for 3 days – TSA Plates

EM Test Method Validation - Plates

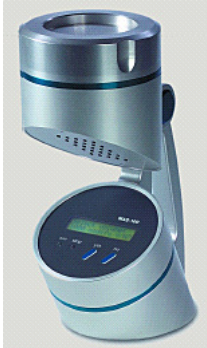
- Required but limited to % recovery and fertility

EM Settle Plates

- Qualify plates per supplier by recovery study > 70% expected. (limits 50% - 200% recovery))
  - Add say 50 - 100 cfu to the plate then count % recovered for a bacteria, a yeast and a mold
- Consider sanitant inhibitor plates
- 4 hour exposure
- Use near expired plates
- Add back specified organisms

EM Test Method Validation – Air Samples

- Required to sample cubing meter of air
- Concerned about plates drying out during exposure giving low recovery
- Validate by
  - Exposure of plate for say 2000 or 3000litres
  - Take to the lab and add back organisms including isolates
  - Expect recovery to be >70%



EM Test Method Validation - Surface

- Required but limited to % recovery and fertility

EM Swabs

- Add say 100 cfu to cleanroom (representative) different surfaces then count % recovered for a bacteria, a yeast and a mold
- Expect >70% recovery (Expect > 50%)
- Are qualifying each “swabber” technique
- Consider sanitant inhibitor – peptone water

Grade A
Critical Space and Critical Surfaces

Critical Space – Grade A / ISO 5

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

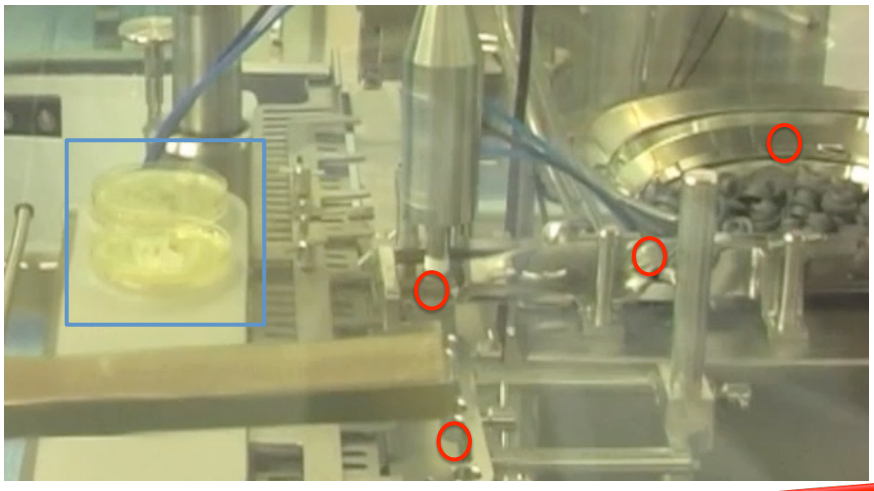
Critical Surfaces within Critical Space

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



Personnel Monitoring Aseptic Operators

Sterile Gowns

- Initial qualification 3 times per operator
- 6 or 12 monthly gowning verification
- End of day surveillance - operators in rotation
- Multiple spots in rotation

Gloves

- Initial qualification
- End of aseptic session / end of shift in rotation
- Post entry into Grade A space for all “high risk” interventions
- Left and right hands – 5 fingers

Gown and Glove Monitoring with Rodacs

Grade A / B Excursions and Investigations

- Sample type - contact plate, settle plate, active air sampler, gown)
- Location of sample (distance to critical surfaces)
- Review of relevant air visualisation / smoke studies
- Microbiological identity to species level
- Review of operations during time period (include operator interview, video review, review of event logs)
- Review of relevant EM data (micro, physical - differential pressure, non viables, temp, humidity)
- Review of trend data (historical and after the event)
- Further investigations in regard of potential sample contamination either during sampling or in the labs
- Specific monitoring programs to support root cause investigations

Crucial information for EM Excursion Risk Assessments

- Is it an isolated event (only 1 sample contaminated)?
- Quantity of contamination (e.g 1 CFU or 25 CFU)
- What is the type of organism ?
- Identification of microorganism (human origin, mold, etc.)
- Plausible most probable root cause(s) informs assessment of product exposure or impact
- Distance to open product and/or critical surface (including airflow pattern)

Usual suspects - sources of organisms

- Personnel ! Operators, cleaners, fitters, transients
- Personnel ! Poor gowning practices
- Personnel ! Poor aseptic techniques / hand sanitisation
- Tracked in via ancillary equipment – trolleys, tanks etc.
- Poor aseptic transfers of materials through pass through etc.
- Inadequate cleaning e.g.. underneath equipment or on conveyors
- Via leaks in HVAC / HEPA system

EM Programs and Sanitation

- EM data monitors the effectiveness of the C&S program
- There is no one magic sanitant so a combination is needed.
  Vegetative cells ➔ Fungal spores ➔ Bacterial spores
  Sanitisers .... Disinfectants ..... Sporicides…....
- EM trend reviews underpin confidence in C & S program and cleanroom management
- Should identify & trend fungi/mold separately to bacteria
- For new sanitant should validate effectiveness “in field”. Sanitant surface residues can inhibit EM growth

Sanitants and Disinfectant Efficacy Studies (USP <1072>)

- Required for all Grade A and B sanitants
- Lab coupon studies (5 x 5 cm) on range of surfaces (glass, vinyl, stainless, EDPM, wrap, plastics etc.)
- Use a full range of micro-organisms - (bacteria, yeast/mold, spore and isolates
- Need to mimic manufacturers methods but worst case application (80% of residence time and aged sanitant)
- > 70% recovery expected
- Should do “insitu” recovery studies as well
- Must have an expiry date for all sanitants
- Required to periodically test sanitants to verify they remain sterile

Flash Quiz

	Environmental Monitoring	Your Selection
1	(a) Grade A airflow pattern studies should be videotaped (b) Videotaping is only needed under “at rest” conditions (c) There should be “alert” and “action EM limits (d) Personnel gloves should be monitored when they conduct an intervention	
2	a) Continuous viable environmental monitoring is required in Grade A (Class 100) areas during operations b) Settle plates are more sensitive than air samples c) Settle plates measure something different to contact plates d) Surface monitoring is not needed if air sampling is used	
3	WHO/FDA/PICs/Japanese EM action limits are almost identical	TRUE/FALSE
4	If a Grade A viable limit is exceeded the batch must be rejected	TRUE/FALSE

Centre for Biopharmaceutical Excellence

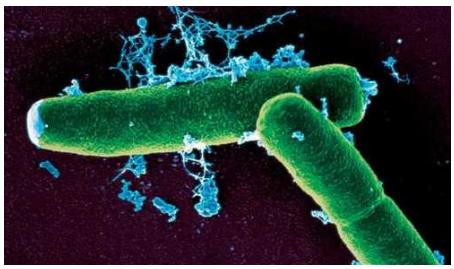
Validation and Management of Heat Sterilization
(Autoclave and Dry Heat Oven)

Useful References

- FDA – Recommendations for Submitting Documentation for Sterilisation Process Validation, November 1994
- ANSI/AAMI/ISO 11134 – Sterilisation of HealthCare products – requirements for validation and routine control – Industrial moist heat sterilisation (1993)
- BP Appendix XVIII Methods of Sterilisation - Monograph for Biological Indicators
- ANSI/AAMI ST79:2006 – Comprehensive guide to steam sterilisation and sterility assurance in health care facilities
- AAMI TIR 13:1997 Principles of industrial moist heat sterilization

How Does An Autoclave Sterilize?

- Steam held at elevated temperature and pressure for time is used to transfer moist heat.
- The steam condenses on a surface and releases energy
- The energy splits open the cell wall.
- Heat acts to denature proteins, effectively killing all cells present.
- Effectiveness is reliant on saturated steam condensing



Define Sterile (IJ Pflug)

Sterile
Free from viable microorganisms.

Sterilisation
Any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms), and inactivates viruses. Therefore the terms "sterile" and "sterilization", in a strictly biological sense, describe the absence or destruction of all viable microorganisms. In other words, they are absolute terms: an object or system is either "sterile" or "not sterile".

The destruction of a microbial population subjected to a sterilization process follows a geometrical progression – to be 100% certain the article is sterile it would require infinite sterilisation.

Sterility Assurance Level (SAL)
For practical purposes the probability of finding a non-sterile unit (PNSU = Probability of Non Sterile Unit) must therefore be lower than 10^{-6}.

Heat Sterilisation Methods

- **Moist Heat (Steam)**
  - Air in autoclave chamber is displaced by saturated steam
  - Condensing water vapour acts as a conductor of heat

- **Dry Heat Oven or Tunnel**
  - Heated dry air is distributed throughout an oven or tunnel by convection or radiation

Industry Rules -Terminal Sterilisation (BP/EP)

- Wherever possible, a process in which the product is sterilised in its final container (terminal sterilisation) is chosen.
- If terminal sterilisation is not possible, filtration through a bacteria-retentive filter or aseptic processing is used;
- Wherever possible, appropriate additional treatment of the product (for example, heating of the product) in its final container is applied.
- In all cases, the container and closure are required to maintain the sterility of the product throughout its shelf-life.

BP/ EP Monograph - XVIII

- Sterility is the absence of viable micro-organisms.
- **The sterility of a product cannot be guaranteed by testing;** it has to be assured by the application of a suitably validated production process.
- It is essential that the effect of the chosen sterilisation procedure on the product (including its final container or package) is investigated to ensure effectiveness and the integrity of the product and that the procedure is validated before being applied in practice
- Revalidation is carried out whenever major changes in the sterilisation procedure, including changes in the load, take place.

Why Are Autoclaves Essential?

- Easiest way to sterilise large volumes of heat tolerant materials.
  - More effective than dry heat (lower temperature /shorter time
  - Not as messy as chemicals and more reliable
  - No need for radiation shielding etc.
- Once validated, simple indicators used to tell autoclaved and non autoclaved material apart – the temp/time/pressure trace is used to confirm sterilization occurred.
- Can deliver > 10^{12} sterility assurance

Definitions: D-Value, Z-Value and Fo

- **What is the D value?**
  - refers to **decimal reduction time** - The time required at a certain temperature to kill 90% (eg reduce population by log 1) of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. Dependant on microbe and initial numbers. Eg D value of 1.5 means it takes 1.5minutes to reduce 1 log (to 10%) @121oC. A Dvalue of 2.0 means more resistant while a Dvalue of 1min means less resistant.

- **What is a Z value?**
  - Refers to the temperature change required to produce a 1 log reduction in D value.

Definitions: D-Value, Z-Value and Fo

What is F_0?

- The number of minutes to kill a specified number of microbes with a Z value of 10ºC at a temp of 121.1ºC.
- Often confused with the time the chamber is held at elevated temperature and pressure and in practice is the same thing.
- Fos accumulate as the sterilisation cycle progresses – very little accumulation below 112ºC.

Overkill

- Use many more microbes than would find on items typically autoclaved. Negates the need to test sample for bioload before running the cycle.
- Use a sterilisation time exceeding what is necessary to kill a large number of microbes. Negates the need to determine D value of microbe.
- Overkill is generally defined as a 12 log reduction in bioload

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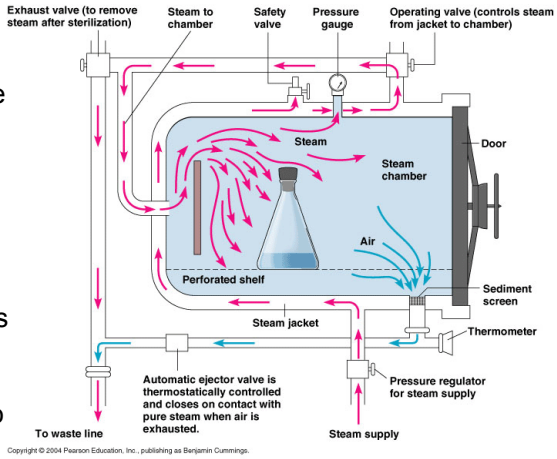
Autoclave Operating Mechanism

Steam enters the chamber jacket, passes through an operating valve and enters the rear of the chamber behind a baffle plate. It flows forward and down through the chamber and the load, exiting at the front bottom.

A pressure regulator maintains jacket and chamber pressure at a minimum of 15 psi, the pressure required for steam to reach 121ºC (250ºF).

Overpressure protection is provided by a safety valve.

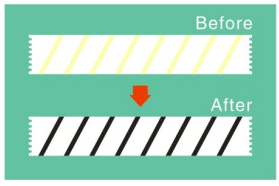


Monitoring of Sterilisation Processes

- **Biological measurements**
  - Required to demonstrate that sterilisation process was **effective**

- **Physical measurements**
  - Time, temperature, pressure, vacuum.
  - Required to **calculate** sterility assurance levels (SAL)

- **Chemical measurements**
  - Autoclave tape or other indicators such as Bowie Dick



Biological Indicators (BIs)

- A **characterized preparation** of a specific microorganism that provides a defined and stable resistance to a specific sterilization process.
- Typically **spore-forming** bacteria
- Used to:
  - Assist in the **PQ** of the sterilization equipment and
  - Assist in the **development and establishment** of a validated sterilization process for a particular article.
  - **Monitor** established sterilization cycles
  - **Periodically revalidate** sterilization processes
  - **Evaluate the capability** of processes used to decontaminate isolators or aseptic clean-room environments.

Examples of Biological Indicators

Sterilisation Method	Organism (Spore type)	Identification	No. Viable Organisms	D value
Steam	Bacillus stearothermophilus Clostridium sporogenes Bacillus subtilis spp	NCTC 10007 NCIB 8157 ATCC 7953 NCTC 8594 NCIB 8053 ATCC 7955	1.0×10^5 to 5.0×10^6 per unit	Typically 1.5 min to 2.5 min @ 121°C
Dry Heat	Bacillus subtilis	NCIB 8058 ATCC 9372	1.0×10^6 to 5.0×10^6 per unit	1min to 3 min @ 160°C Typically 1.9 min @ 160°C
Radiation	Bacillus pumilus (min. dose of 25kGy) Bacillus cereus (for higher dose levels)	NCTC 824 NCIB 8982 ATCC 14884 SSI C 1/1	$>10^7$ - 10^8 per indicator unit	~3 kGy (0.3 MRad)
Ethylene Oxide	Bacillus subtilis, variety Niger	NCTC 10073 ATCC 9372	1.0×10^6 to 5.0×10^7 per unit	2.5 min to 5.8 min @ ETO 600mg/l 60% RH and 54°C Typically 3.5
Filtration	Pseudomonas diminuta	ATCC 19146	recommend $\geq10^7$	NA

Example Dvalues of Organisms

AVERAGE VALUES OF D AND Z FOR SOME REPRESENTATIVE MICROORGANISMS Wallhauser 1980

Microorganism	D_{121}	z
Clostridium botulinum	0.2	10
Bacillus stearothermophilus	2.0	6
Bacillus subtilis	0.5	10
Bacillus megaterium	0.04	7
Bacillus cereus	0.007	10
Clostridium sporogenes	0.8 - 1.4	13
Clostridium histolyticum	0.01	10

F_o Calculations – BP/EP

$$F_o = D_{121}(\text{Log } N_o - \text{Log } N) = D_{121}\text{Log IF}$$

D_{121} = D-value of the reference spores (5.1.2) at 121 °C,
N_0 = initial number of viable micro-organisms,
N = final number of viable micro-organisms,
IF = inactivation factor.

$$IF = N_o/N = 10^{\,t/D}$$

t = exposure time
D = D-value of micro-organism in the exposure conditions.

TABLE OF LETHAL RATIOS

WHOLE °C	TEMPERATURES + TENTHS OF A°C									
	0.	1.	2.	3.	4.	5.	6.	7.	8.	9.
105	.024	.025	.026	.026	.027	.027	.028	.029	.029	.030
106	.031	.032	.032	.033	.034	.035	.035	.036	.037	.038
107	.039	.040	.041	.042	.043	.044	.045	.046	.047	.048
108	.049	.050	.051	.052	.054	.055	.056	.057	.059	.060
109	.062	.063	.064	.066	.067	.069	.071	.072	.074	.076
110	.077	.079	.081	.083	.085	.087	.089	.091	.093	.095
111	.097	.100	.102	.104	.107	.109	.112	.115	.117	.120
112	.123	.126	.128	.131	.135	.138	.141	.144	.148	.151
113	.154	.158	.162	.166	.169	.173	.177	.182	.186	.190
114	.194	.199	.204	.208	.213	.218	.223	.229	.234	.239
115	.245	.251	.256	.262	.268	.275	.281	.288	.294	.301
116	.308	.315	.323	.330	.338	.346	.354	.362	.371	.379
117	.338	.397	.406	.416	.426	.435	.446	.456	.467	.477
118	.489	.500	.512	.523	.536	.548	.561	.574	.587	.601
119	.615	.629	.644	.659	.674	.690	.706	.723	.739	.757
120	.774	.792	.881	.830	.849	.869	.889	.910	.931	.953
121	.975	.997	1.021	1.044	1.069	1.093	1.119	1.145	1.172	1.199
122	1.227	1.256	1.285	1.315	1.346	1.377	1.409	1.442	1.475	1.510
123	1.545	1.581	1.618	1.655	1.694	1.733	1.774	1.815	1.857	1.901
124	1.945	1.990	2.037	2.084	2.133	2.182	2.223	2.285	2.338	2.393
125	2.448	2.506	2.564	2.624	2.685	2.747	2.811	2.877	2.994	3.012
126	3.082	3.154	3.228	3.303	3.380	3.459	3.539	3.622	3.706	3.792
127	3.881	3.971	4.063	4.158	4.255	4.354	4.456	4.559	4.666	4.774
128	4.885	4.999	5.116	5.235	5.357	5.482	5.608	5.740	5.874	6.010
129	6.150	6.294	6.440	6.590	6.744	6.901	7.062	7.226	7.394	7.567
130	7.743	7.293	8.108	8.297	8.490	8.668	8.890	9.097	9.309	9.526

F_o Tables

Points to Note

1. 121.1 = Fo of 1min
2. Below around 112 very little accumulated Fos
3. Increase/decrease is exponential … slight changes have a big impact.
4. The F_0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process

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PNSU, SAL and Overkill

- Sterility assurance level (SAL) is the reciprocal of Probability of a Non-Sterile Unit (PNSU).
- The purpose of a BI challenge is to establish that the biological lethality is equivalent to the physically determined F_0, generally measured by thermocouples.
- **SAL = F_o / D_{value}**
  - With a D_{value} of 1.5min and a F_o of 18_{min} = we have an 12 log reduction. If we started with 10^6 we would end up with 10^{-6} which is the PNSU so we have an SAL of 10^{12}
- "Overkill" generally means that you develop a cycle that gives a complete kill of BIs with a N_o of 10^6 and then you double that cycle – otherwise can use a reduced cycle approach – Overkill is really over overkill and only suitable for equipment.

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Critical parameters needed for successful sterilization

- Article wrapping
- Chamber load pattern
- Air removal (steam displacement or vacuum)
- Moisture (saturated steam)
- Pressure / vacuum conditions
- Temperature
- Cycle Time and “Dwell” Time
- Contact with surfaces:
  - Packaging permeable to moist heat
  - Items designed to allow contact
  - Items designed to allow air removal

What Can Go Wrong ?

- Effective sterilization is dependant on:
  - initial bioload of incoming materials
  - Microbe resistance to heat (D_{value}) of that bioburden
  - Time the autoclave is held at a sterilizing temperature
  - Ability of steam to penetrate items being sterilized

Steam Penetration:

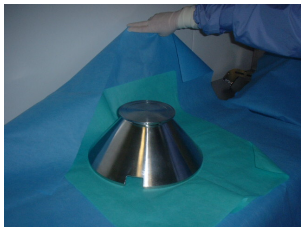
As steam is used to transfer heat, tightly wrapped items, or long tubing may not be properly penetrated. Would represent worse case for validation.

Air Pockets:

Trapped air creates localised dry heat conditions – reducing lethality rates

Wrapping Articles and Load Descriptions
(Must develop equipment wrapping program)

- Must completely seal the wrap
- Generally 2 - 3 sealed layers
- Overwrapped articles retain moisture
- Must include BI and T/C when validating article
- Must specify load in autoclave
  - Number and type of artlcles
  - Specific location (diagram / photo)
  - **Load pattern must appear in operating procedure**



The Problem of Air

- Pockets of trapped air result in localized dry heat conditions which reduces the SAL.
- Autoclaves without vacuum are considered “non-GMP”
- Air removal relies on
  - Vacuum pre-pulsing the chamber before introduction of steam – generally 3 - 4 times
  - Careful consideration of the load pattern and contents
- Known issues with air removal:
  - Extended length of transfer tubing
  - Filters mis-orientated to trap air
  - Tank valves closed off to prevent removal
- Air inlet at end of the cycle must be sterilized via an air filter – filter must be periodically integrity tested.

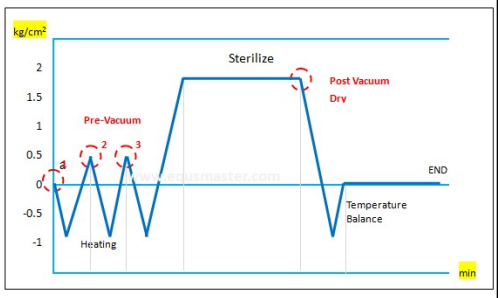
Steam Supply Quality

- Expected to test steam quality regularly = WFI minus bioload.
- HTM-2010 (UK Standard) sets our requirements for steam quality wrt validation and monitoring
- HSA Guidance states “Steam quality must be tested periodically to ensure that:
  - moist heat (rather than dry-heat) sterilising conditions are achieved;
  - superheating does not occur;
  - wet loads are avoided;
  - non-condensable gases is below 3.5%; and
  - mineral and organic impurities (including bacteria and pyrogens) are below specified maximum levels.

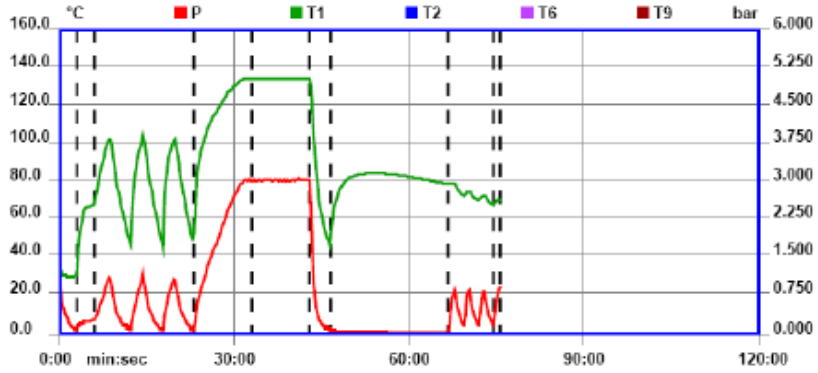
The three basic steam quality tests are the superheat test, dryness value and non-condensable gas tests.

Operating Characteristics of Steam Sterilisers

- Air Removal Options
  - ✗ **Gravity displacement:**
    - Steam enters and displaces the residual air through an open vent
  - ✔ **Vacuum air removal:**
    - Air is removed with a mechanical pump prior to dwell time.
- Pressure is needed to achieve high temperatures (steam)
- Must release pressure slowly for liquids (slow exhaust)
- Items must be allowed to dry before removal from chamber



Example time/temperature/pressure Print-off.

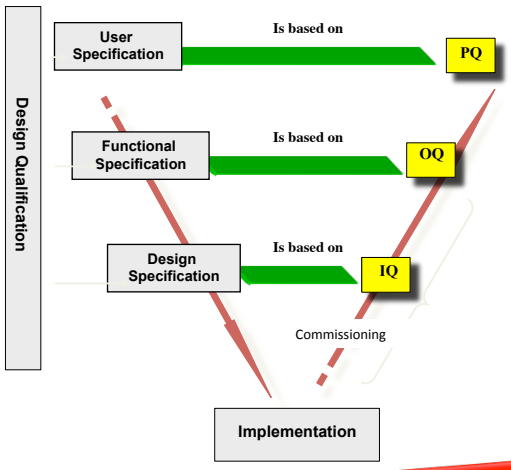


Validation Principles

- The basic principles for validation of a heat sterilizing process are:
  - Must use BIs to demonstrate lethality
  - Must use thermometrics/ thermocouples
  - Cycle development and description of load patterns are pre-requisites
  - Can do time/temperature or Fo approach for control
  - Calibrate thermocouples both pre and again post
  - Must include **"worst case"** conditions
    - Maximum and minimum loads/ patterns
    - One run of reduced cycle time / temperature
    - Cold start for at least one of three runs per load pattern

Validation Approach and Sequences

- **DQ:** Has the item been specified correctly ?
- **IQ:** does equipment meet the URS requirements? Is everything that was on the box, in the box? Is the unit installed properly. Are support programs in place for ongoing operation of A/C?
- **OQ**: does the A/C operate properly? Does the unit hold temp and pressure correctly?
- **PQ**: validation of autoclave cycles and loading patterns – need to show sterilization.



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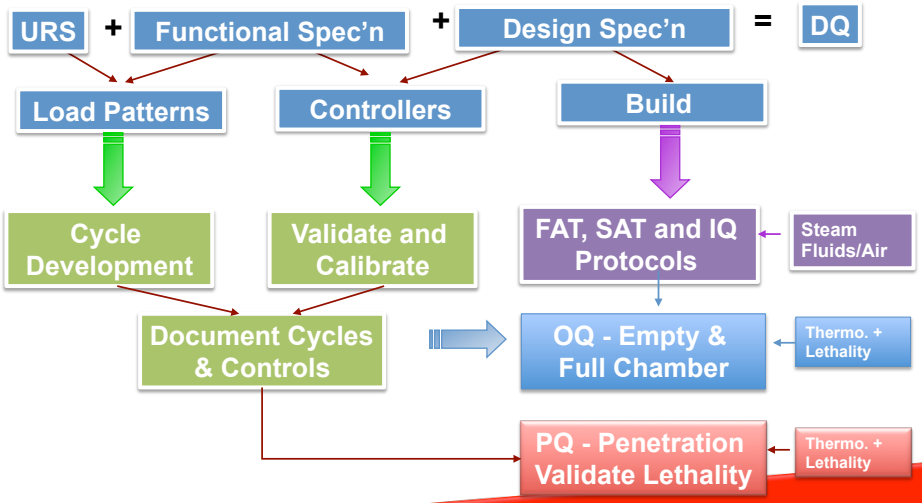
Overview of Sterilisation Validation
(Scope of Works)



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Validating Load Patterns
(Why are load patterns important?)

- Sterilization relies on steam penetration. Need to validate each load patterns as steam distribution may vary;
- Loading patterns should be documented and adhered to.
  - Worse case validated – can use less but not more equipment
- BIs: When to use spore strips and when to use solutions
- **How to validate?**
  - 3x successful runs each loading pattern
  - Place BI with each item in worse case spot. Place thermocouple next to BI, but not touching item.
- How often to re qualify? – annually expected

Autoclave - Operational Qualification

- **Empty Chamber Thermal Mapping**
  - Verify the heat distribution pattern in an empty chamber
  - Repeat annually to re-confirm operation of autoclave
  - Conduct cold start and hot start
- **Controller Reliability**
  - Ensure each step in the PLC is in the correct sequence and is repeatable. Failure modes should include failure and restart of the critical services and include:
  - Electrical power loss,
  - Loss of equipment or instrument compressed air loss,
  - Service loss: jacket or pure steam, cooling water, vacuum,
  - Other critical service.

Operational Qualification – Control Systems

- **Control System Verification**:
  - Sterile Door Security,
  - Program Change/Alteration Security,
  - Cycle program Back Up and Recovery,
  - Calculation of F_0 Accuracy,
  - Independence of Controlling and Monitoring Thermocouples,
  - Accuracy of Printout Record.
- **Alarm and display indicators**,
  - Ensure these indicate the correct status of the autoclave for each cycle,
- **Door Interlock**
  - must work correctly not allowing access during the cycle,
- **Gasket Integrity/ Leak testing**
  - Verify positive/negative pressure seal of all door gaskets.
  - Bowie Dick Test to demonstrate air removal from chamber

PQ of Autoclave

- PQ: validation of autoclave cycles and loading patterns.
- What SAL do you need?
  - Need to show a 10^6 or 10^{12} reduction of microbes.
- What is your starting bioload?
  - Spore strips have >10^6 CFU.
- What is the microbe's D value?
  - For *Geobacillus stearothermophilus*, this is around 1.5 – 2.0
- Must use physical, chemical and biological indicators (Bis).

PQ of Autoclave

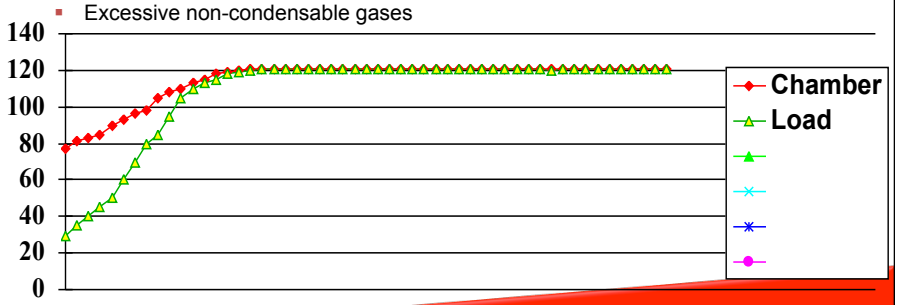
- **Heat Distribution Study** – how does steam circulate around the contents ? Is it consistent ? Can be done with thermocouples only.
- **Heat Penetration Study** – how quickly does the heat penetrate the item or liquid.
  - Maximum Loads
  - Minimum Loads – what does this mean ?
- **Worst Case Conditions**
  - Reduced time and temperature
  - If overkill needed 50% of cycle to show $>10^{-6}$ – production cycle is doubled to achieve 12 log reduction.

Performance Qualification
(Heat Penetration Studies)

- **Heat penetration studies** – carried out for each load configuration for each nominated cycle with the aim to:
  - Identify any cold spots within the load;
  - Measure the accumulated F_o for each challenge location within the nominated load.
- Microbiological challenge (lethality) studies carried out as part of heat penetration studies (reduced exposure).
- Product degradation (maximum exposure)
- Load "lag time" or come up determination – look for slowest to heat location
- BI is Geobacillus stearothermophilus with a certified D-value between 1.5 and 2.0 and a verified spore count of between $5x10^5$ and $5x10^6$,

Load Equilibration Time

- Equilibration time, that is, the time for the penetration thermocouples to show the same temperature as the chamber.
- Ideally equilibration time should be less than 15 seconds for chambers less than 800Litres and 30 seconds for larger chambers.
- If the equilibration time is exceeded it diagnoses:
  - Inadequate air removal OR
  - Inadequate steam penetration OR
  - Excessive non-condensable gases



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Example Acceptance Criteria (Equipment Load)

- Four pulses of vacuum down to 25 kPa
- 3 positive pulses of steam to 160 kPa
- Sterilisation set-point temperature 124°C for lowest T/C
- All T/Cs within range 124°C -126°C during dwell
- T/C does not fluctuate by > 1°C during dwell
- Sterilisation dwell time 15 minutes
- Accumulate > 30 F_o
- All Bis show no growth
- Post sterilisation drying time 20 minutes
- Leak rate tests remain within specification
- At least 9 of 10 T/Cs remain within calibration

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Annual Re-validation Example (Include the following tests)

1 Chamber leak rate test
2. Air removal and steam penetration test (Bowie Dick Test)
3. Heat distribution studies for empty chamber (1x)
4. Heat penetration studies for standard production loads:
   - Load #1 Filling Components
   - Load #2 Filling Machine Cap Components
   - Load #3 Filling Machine Stopper Components

5. Biological challenge testing for standard loads
6. Steam condensate quality test
7. Planned preventative maintenance schedule, including instrument calibration

"Three consecutive cycles shall be tested for each load configuration to demonstrate consistency of autoclave performance".

Routine Monitoring of Autoclaves

- Sequential number runs and a running log
- Must double sign prints to verify cycle conditions met
- Record conditions met and any alarms activated
- Chamber Leak Rate Test (weekly)
- Physical indicator on each item in each load
- Bowie Dick Test (Optional)
- BIs are not routinely included in the cycle
- Reliance on controlling probe (directly correlated to the worst case (coldest) location for the validation probe
- For product loads usual ot probe a number of dummy vials in the load for added assurance.

Auditor Considerations

What do GMP auditors look for in an audit

- Was re-validation conducted in time frame?
- Focus on PQ primarily but interest in IQ/OQ for newer autoclaves
- Coolest and warmest positions clearly stated in validation report?
- Preventative maintenance program, SOPS, leak rate test data ?
- Cycle time / Fo – is it sufficient for tested D values?
- Was validation equipment within calibration (pre and post use))
- Traces for validation and most recent cycles – consistency ?
- Are vacuum cycles used appropriately?
- Is anything thing not listed on the loading pattern present in the autoclave? Enough room for steam to circulate through chamber?
- Deviations from protocols. Are conclusions valid and justified?
- Can site demonstrate terminally sterilised product is stable?

USP <1211>
Dry-Heat Sterilization/Depyrogenation

- A dry-heat sterilization/depyrogenation system is supplied with heated, HEPA filtered air, distributed uniformly throughout the unit by convection or radiation and employing a blower system with devices for sensing, monitoring, and controlling all critical parameters.

- A typical acceptable range in temperature in the empty chamber is ±15oC when the unit is operating at not less than 250oC .

Depyrogenation of Glassware

- Dry heat is used for depyrogenation purposes and results in complete destruction of micro-organisms
- It is accepted that validation of depyrogenation means also SALs much greater than 10^{-6}.
- Dry heat at temperatures greater than 220°C is frequently used for sterilisation and depyrogenation of glassware. In this case demonstration of a 3-log reduction in heat resistant endotoxin can be used as a replacement for biological indicators. (BP/EP)
- Spores of Bacillus subtilis (for example, var. niger ATCC 9372, NCIMB 8058 or CIP 77.18) are recommended as biological indicators.

Example of Depyrogenation Cycle Description

Cycle phase description	Set-point
Dehumidifying Rate:	6.0°C/min
Dehumidifying Time:	45 minutes
Dehumidifying Temperature:	120°C
Exposure Rate:	5.0°C/min
Exposure Time:	195 min
Exposure Temperature:	245°C
Cool Down Rate:	2.0°C/min
Cool Down Temperature:	50°C

Also need

- Load Pattern Description
- Location of T/Cs throughout the chamber
- Cycle ranges for parameters

HAO Performance Qualification for Depyrogenation

- Expected to apply endotoxin the inside of glass vials
- Techniques and methods for recovering and testing endotoxin must be validated.
- should recover a minimum of 50% of applied endotoxin from glass surfaces.
- Recovery studies should be performed at the level of expected endotoxin.
- Need to challenge with >10,000 Endotoxin Units (EUs)
- Acceptance criteria is > 3 log reduction demonstrated on 3 consecutive runs for each load pattern.

Example Acceptance Criteria for HAO

Cycle Conditions

Must meet the nominated ranges of the cycle conditions

Thermometrics

- All thermocouple locations shall indicate temperatures continuously in excess of 220oC for a period of at least 2 hours 15 minutes, during the exposure phase of the cycle.
- The timing of the exposure phase of the cycle starts from the slowest to heat thermocouple reaching 220oC and finishes with the fastest to cool thermocouple falling below 220oC.

"Pyrometrics" - > 3 log reduction

Flash Quiz

	Sterilisation	Your Selection
1	(a) Modern autoclaves do not require pre-vacuum systems (b) Heat distribution for loaded chambers is expected at OQ or PQ (c) GMPs require maximum and minimum loads to be validated (d) GMPs do not require reduced cycles if a maximum load is validated	
2	a) Biological indicators (BIs) are not needed for validating depyrogenation ovens b) BIs are expected to be placed in each production autoclave load c) The incoming equipment bioburden does not matter for overkill cycles d) Chamber leak tests need only occur on annual re-validation	
3	The "equilibration" time gives a good clue to the presence of residual air in an autoclave	TRUE/FALSE
4	The presence of saturated steam is a critical factor in effective autoclave operation	TRUE/FALSE

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