Microbiological validation: equipment and operators

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Useful Definitions

❖ Process validation

❖ The documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce a medicinal product meeting its predetermined specifications and quality attributes.

GMP PIC/S - EU
Useful Definitions

Microbiological Monitoring

- Microbiological monitoring is the responsibility of the pharmaceutical manufacturer. It may include environmental monitoring where product is manufactured.

Media fill test (MFT)

- Method of evaluating an aseptic process using a microbial growth medium (Media fills are synonymous to simulated product fills, broth trials, broth fills etc.).
Basis

- Hot topics in the inspection and GMP compliance of sterile production of drugs either in industrial pharmaceutical or in hospital pharmacy.
- The microbiological validation of the different sterile and aseptic production equipments are now unavoidable.
- The media fill tests and the microbiological validation of the operators in the hospital pharmacy is becoming also part of the standard operating procedures.

Cleanrooms Microbiological Monitoring
**Air Viable-Particles Monitoring**

- **Settle plates:**
  - Culture media agar plates placed open during production process
- **Bio-impactor:**
  - Air hovered and accelerated by a fan on a culture medium plate
- **Filtration method:**
  - Air filtered on a porous or agar media retaining microorganisms and setting this sampling support on a culture media plate
- **Results expressed in CFU/plate**

**Surface monitoring**

- **Count-tact® plates:**
  - Flat surfaces, without any roughness
- **Swab sampling:**
  - Uneven surfaces, corners
  - Transfer on culture media plates
Operators’ Gloves monitoring

- Print of the five fingers laid gently on a blood culture plate

Two types of Monitoring

- In Process Monitoring
  - Define the critical points to monitor
  - Define ALERT (Re-Control) & ALARM (Action) limits
  - To not interfere with the process or add any site contamination

- « At REST » Monitoring

S. Fleury, HUG Pharmacy Course, 2008
**In Process Monitoring (PIC/S & BPF)**

<table>
<thead>
<tr>
<th>Air</th>
<th>Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-viables P.</td>
<td>Viable P.</td>
</tr>
<tr>
<td>0.5 µm</td>
<td>5 µm</td>
</tr>
</tbody>
</table>
| A | < 3500 | 0 | < 1 | < 1 | 1
| B | < 350000 | < 2000 | < 10 | < 5 | 5
| C | < 3500000 | < 20000 | < 100 | < 50 | 50
| D | n.d. | n.d. | < 200 | < 100 | 100

**Operators: gloves**

<table>
<thead>
<tr>
<th>CFU/Glove</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>B</td>
</tr>
<tr>
<td>C</td>
</tr>
<tr>
<td>D</td>
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**« At Rest » Monitoring (PIC/S & BPF)**

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**Routine monitoring set to avoid Contamination**
Monitoring: Critical points definition

Specifications Definition

- Type of monitoring operation
  - Surfaces, air, operator
- Place of monitoring
  - Cleanrooms, LAFH, isolator, ...
- Frequency and time
  - In Process, At Rest
  - Every day, week, month, ...
- Alert limits: Re-Controle (e.g. 2/3 of alarm limits)
- Alarm limits: Action (GMP limits or experience based limits)
- Viable and non-viable Particles (GMP limits)
## Decision Tree

### Biocontamination des surfaces

<table>
<thead>
<tr>
<th>Activités</th>
<th>vae</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non conforme</td>
<td>Test de biocontamination des surfaces non conforme</td>
<td></td>
</tr>
<tr>
<td>Nettoyage et désinfection</td>
<td>Aspirer les parties impropres, hydrater</td>
<td></td>
</tr>
<tr>
<td>Conforme</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Non conforme</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Mise à blanc</td>
<td>Nettoyage à fond, y compris murs, plafonds</td>
<td></td>
</tr>
<tr>
<td>Contrôle complet</td>
<td>Contrôle de biocontamination des surfaces, remove de particules</td>
<td></td>
</tr>
<tr>
<td>Resultat conforme</td>
<td>OK</td>
<td></td>
</tr>
<tr>
<td>Resultat non conforme</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test de microbiological validation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Equipment microbiological validation

**Example:**

**Negative Pressure Isolator**
**Basis**

- A Negative Pressure Isolator (Barrier LAFH/BSC Type III) is a closed system essentially used for the preparation of injectable cytotoxic drugs
- This equipment offers a good protection to the operators and to the preparation
- All preparations have to be in accordance with GMPs or simply with Phar. Eur. as a sterile product, confirmed with a validated SAL

**Goals**

- To validate the working procedure (material entry into the isolator and Media fill test)
  - Air sampling with a bio-impactor on culture media plates
  - Surface sampling with Count-tact® plates
  - Sampling with swabs and transferred on plates
  - TSB for MFT, validating the process
  - Operators Gloves sampling on blood plates
**Methodology**

- **Sampling plan**
  - A : Sc1 - Sc4 et C1 - C9 on flat surfaces
  - B : Sp1 - Sp4 ; P1 - P9 ; L1 - L4 et V1 - V3 on vertical lateral walls

- **Swabs sampling plan**
  - E1, E2, E3, E4 : sampling inside the isolator working chamber

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**Sampling Plan**

- I1: Left Airlock
- I2: Isolator front face
- I3: Right Airlock

**Bio-impactor samplings**

M. Ackerman, F. Sadeghipour & al., GSASA Congress, St. Gallen, 2003
**Sampling Plan**

<table>
<thead>
<tr>
<th></th>
<th>C1</th>
<th>C4</th>
<th>C7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc1</td>
<td>C2</td>
<td>C5</td>
<td>C8</td>
</tr>
<tr>
<td>Sc2</td>
<td>C3</td>
<td>C6</td>
<td>C9</td>
</tr>
</tbody>
</table>

Left Airlock

Isolator front face

Right Airlock

Count-tact® samplings

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**Results**

- If any positive result:
  - Identify the reason
  - A total cleaning and disinfection of the isolator
  - Restart the validation until having 3 successive compliant results

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M. Ackerman, F. Sadeghipour & al., GSASA Congress, St. Gallen, 2003
Aseptic Process Validation

Goals

- Validate the aseptic process
- Evaluate the risk to produce non-sterile units
- Evaluate the personnel training in aspetic work
### Micobiological Culture Media

- **Trypcase – Soja Broth (TSB):**
  - Aerobic microorganisms (Bacteria and Moulds) and some anaerobic
- **Thioglycolate:**
  - Anaerobic microorganisms and some Aerobics

### Important properties:
- Fertility et capacity to reveal low contaminations
- Aptitude to sterilization by filtration
- To be clear and limpid to avoid any false positive and identification and scanning artifact
- Low viscosity to ease the transfer and to avoid the stop during filtration
- Sterility
**Fertility testing I**

- Thioglycolate
  - USP
    - Bacillus subtilis (ATCC 6633) *Candida albicans* (ATCC10231), *Bacteroides vulgatus* (ATCC 8482)
  - Phar Eur
    - *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Clostridium sporogenes* (ATCC 19404)

*Other organisms proved to be present in the clean rooms could be used as real and practical species.*

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**Fertility testing II**

- TSB
  - USP
    - Bacillus subtilis (ATCC 6633), *Candida albicans* (ATCC10231)
  - Phar Eur
    - *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404)

*Other organisms proved to be present in the clean rooms could be used as real and practical species.*
Incubation conditions

- 2 Weeks:
  - 1 week: Room temperature (Moulds)
  - 1 week: 35 °C (Bacteria)

The incubation conditions could be modified according to the different types of microorganisms, the bioburden and the environment.

Precautions

- To Avoid False Positives
  - Respect strict aseptic conditions
  - The MFT containers have to be airtight

- To Avoid False Negatives
  - All the internal surfaces have to be “ licked” to be in contact with the culture media
  - Avoid any contamination with disinfectants
  - For TSB, introduce sterile air into the final container for aerobic organisms
  - Respect very strictly the incubation periods
**Reading and Identification**

- Each MFT container is read individually
- If the final container is opaque, transfer at the end of the incubation period into a clear and transparent container
- Detection: under an artificial light and compared to Negative and positive control samples
- The presence of any spot or filament have to be considered as Positive
- The personnel involved in identification has to be trained specifically for this activity
- Any Positive result: microorganism identification

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**Worst-case Conditions**

- Important element of all validations but especially for MFT in order to include unfavorable conditions while approaching as far as possible « normal conditions » of the process.
- The worst case conditions must respect GMPs
- The worst case conditions are coming from the daily practice experiences and are introduced in the different steps of the process to induce difficult conditions for the operator and the aseptic preparations
- Taking into account all the possible problems happening during a process simultaneously throughout a MFT to permit a decision if a minor deviation is occurring during a real production.
MFT acceptance criteria

- A minimum of 3 consecutive conform tests are mandatory to validate an Aseptic Process.
- The batch size of units filled for a MFT depends on an usual batch size (ISO 13408-1):
  - **Hospitals**:
    - batch size is representative of daily batch sizes: Small batch sizes
    - Number of MFT: depends of the type of different processes used

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MFT acceptance criteria

**Industry**:
Positives: \[ \leq 0.1\% \text{ of MFT units} \]
0 if \(< 3000 \text{ units}\)

Contamination rate = Upper 95\% confidence limit \times 100\%
Number of filled units

**Hospital**:
0 !!!

PIC/S PI007-2 Recommendation on the validation of aseptic processes
MFT : special considerations

- Any **must** to be considered as a
  - Critical Alarm Signal for any batch size
  - Fix Alarm and Action Levels

- To tend to **0** for any batch size

Validation Elements

- A new operator
- A new equipment
- Any operator or equipment not operating since 12 months
- A *New Process* or after any *Major Change*
- The Revalidation of any process which is not controlled totally anymore
### Periodic MFT (industry)

- 2 MFT / year
- 1 MFT after any process interruption because of a microbiological problem
- Any *major deviation*

### Simulation elements (industry)

<table>
<thead>
<tr>
<th>Routine steps <em>(systematically)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Team change</td>
</tr>
<tr>
<td>Changes in primary packaging (vials, stoppers)</td>
</tr>
<tr>
<td>Any change in filling vessels</td>
</tr>
<tr>
<td>Sampling process</td>
</tr>
<tr>
<td>New manipulation or adjustment during the aseptic process</td>
</tr>
<tr>
<td>Environment monitoring and IPC</td>
</tr>
<tr>
<td>Changes in the transfer of the filled vials for stoppering, crimping</td>
</tr>
<tr>
<td>Stopping and restarting the equipment after an operator intervention during filling process</td>
</tr>
</tbody>
</table>
### Simulation elements *(industry)*

<table>
<thead>
<tr>
<th>Exceptional process elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>A maintenance intervention</td>
</tr>
<tr>
<td>Changing an accessory (filter, gloves, purging the system)</td>
</tr>
<tr>
<td>Cleaning intervention</td>
</tr>
<tr>
<td>Modification of the environment conditions (overpressure change in the limits)</td>
</tr>
</tbody>
</table>

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### Operators Validation

C. Stucki, I. de Giorgi, HUG Pharmacy Course, 2008
Goals

- The aseptic operations depend mainly on the TRAINING, KNOW-HOW, and the behavior of the operator.
- MFT protocols are adapted to the procedures of each production site.

MFT Protocols

- Evaluate the Operator capacity to maintain the sterility of the preparation during the aseptic process.
- Standardized validation:
  - Initial validation for each new operator
  - Periodic validation for operators.
Validation Protocol

- The MFT is validated if 3 successive conform tests are successful for each new operator.
- A periodic validation is scheduled once a year for each operator.
- Each operator performs 4 different types of preparation in different existing production environments.

General Conditions

- Examples of type protocols to consider environmental conditions:
  - Horizontal laminar airflow hood H-LAFH
  - Vertical laminar airflow hood V-LAFH or BSC (BioSafety Cabinets Type II)
  - Negative pressure isolator/Barrier LAFH (BSC Type III)
**Worst-Case Conditions**

- The total time for different types of fillings
- Presence of the Validation Officer
- Schedule the MFT at the end of the work session (tiredness)
- The installation of all the materials by the operator without any intervention of the Validation Officer

**Outcomes**

- The understanding of the operators about the usefulness of the MFT is an essential element of the success of these validations
- To consider that to validate a whole team is very time and resources consuming
- It is simultaneously an excellent opportunity to draw operators attention on Contamination control
General Conclusions

- Sterile drugs by Aseptic techniques and maintaining GMP-compliant cleanrooms are an everyday challenge.
- The only way to cover the maximum of risks is to have a robust Quality assurance system.
- The Sterility is assured with the combination of:
  - Regular and structured Monitoring of the cleanrooms
  - Validation of the production equipments (LAFH, Isolators)
  - Aseptic Process validation by MFT, especially for batch production
  - Validation of the operators by simple protocols based on usual procedures
    - Microbiological
    - Chemical

MFT References

- USP Chap 797: personnel validation
- BPP (F): Process validation
- EC Guide to GMP for medicinal products and active pharmaceutical ingredients, annex 1, Rev 1996
- FDA Guidance for industry - Sterile Drug products produced by aseptic processing
- Pharmaceutical CGMPs (2004)
- PIC/S 007: Recommendations on the validation of aseptic processes (2001)
- Bussières JF, Mise en place d’un protocole de validation microbiologique en hémato-oncologie, Pharmactuel Vol. 39 N° 4 Août - Septembre 2006