Microbiological validation: equipment and operators

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EAHP Foundation Seminar:
"Patient Safety; More About Compounding"
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Krakow, Poland

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.

GMP PIC/S - EU

Useful Definitions

- 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.).

GMP PIC/S - EU

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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

Pharmacie

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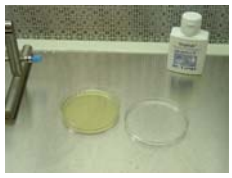
Air Viable-Particles Monitoring

- Settle plates :
  - Culture media agar plates placed open during production process

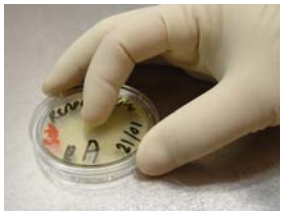


- Bio-impactor :
  - Air hovered and accelerated by a fan on a culture medium plate

- 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

« At REST » 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

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In Process Monitoring
(PIC/S & BPF)

Air

	Non-viables P.		Viable P.	
	0.5 µm	5 µm	CFU/m³	CFU/4h
A	< 3500	0	< 1	< 1
B	< 350000	< 2000	< 10	< 5
C	< 3500000	< 20000	< 100	< 50
D	n.d.	n.d.	< 200	< 100

Surfaces

	CFU/ Plate
A	< 1
B	< 5
C	< 25
D	< 50

Operators : gloves

	CFU/Glove
A	< 1
B	< 5
C	-
D	-

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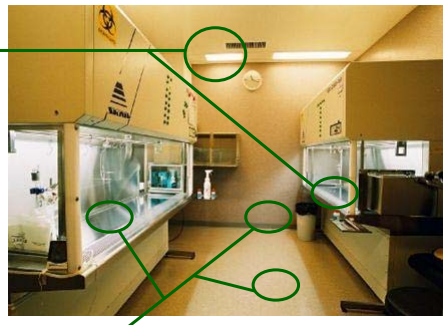
« At Rest » Monitoring
(PIC/S & BPF)

Air

	Non-viables P.		Viable P.	
	0.5 µm	5 µm	CFU/m³	CFU/4h
A	< 3500	0	< 1	< 1
B	< 3500	0	< 10	< 5
C	< 350000	< 2000	< 100	< 50
D	<3500000	< 20000	< 200	< 100



Surfaces

	CFU/ Plate
A	< 1
B	< 5
C	< 25
D	< 50

Routine monitoring set
to avoid **Contamination**

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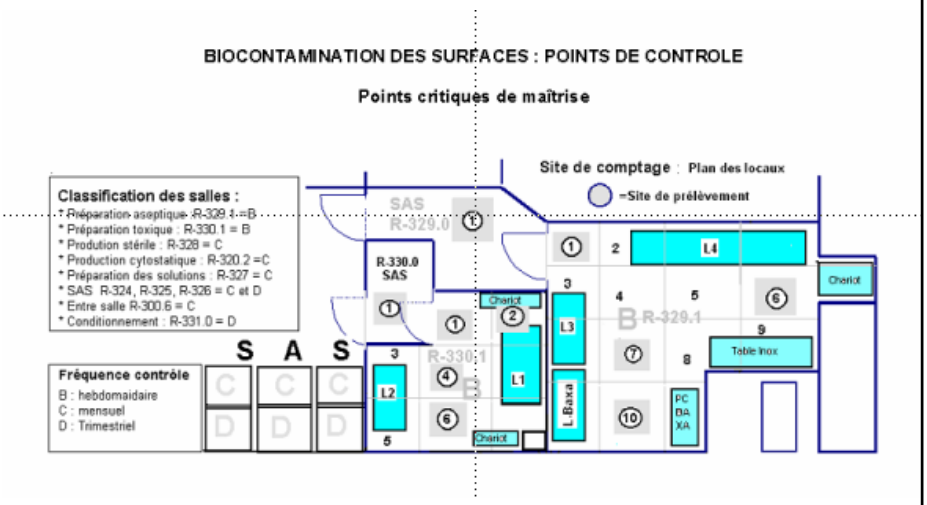
Monitoring : Critical points definition



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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)

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Decision Tree

Biocontamination des surfaces		
Activités	**vers**	Descriptiona
Non conforme		Test de biocontamination des surfaces non-conforme
↓ Nettoyage mécanique		Avertir le service Propreté-Hygiène
↓ Contrôle 3 j d'affilée		
↓ Conforme	**OK**	
↓ Non conforme	**2**	
↓ Mise à blanc		Nettoyage à fond, y compris murs, plafonds
↓ Contrôle complet		Contrôle de : Aérobiocontamination Biocontamination des surfaces Comptage de particules
↓ Résultat conforme	**OK**	
↓ Résultat non conforme		
↓ Fermeture du local		
↓ Cellule de crise		

Equipment microbiological validation

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Example : Negative Pressure Isolator

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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

M. Ackerman, F. Sadeghipour & al., GSASA Congress, St- Gallen, 2003

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

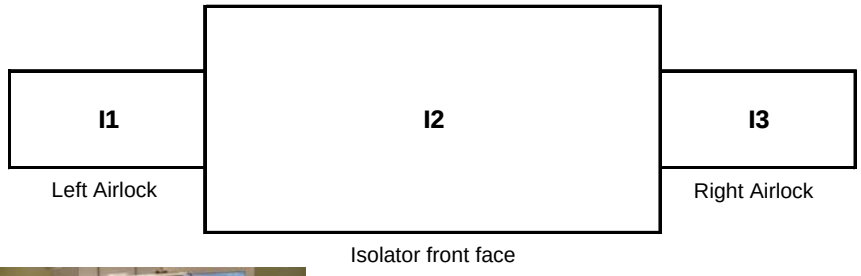
M. Ackerman, F. Sadeghipour & al., GSASA Congress, St- Gallen, 2003

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

M. Ackerman, F. Sadeghipour & al., GSASA Congress, St- Gallen, 2003

Sampling Plan



Bio-impactor samplings

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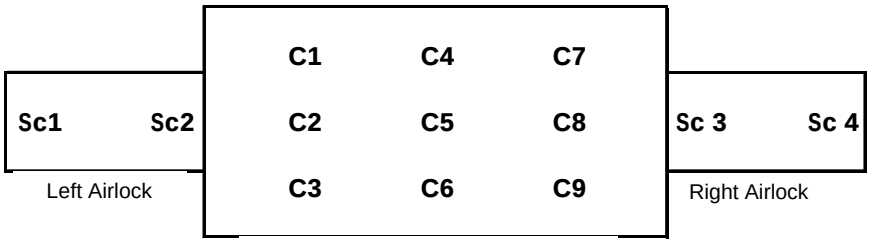
Sampling Plan



Count-tact® samplings

M. Ackerman, F. Sadeghipour & al., GSASA Congress, St- Gallen, 2003

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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Aseptic Process Validation

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Goals

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

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Micobiological Culture Media

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

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Micobiological Culture Media

- 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

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Fertility testing I

- Thioglycolate
  - USP
    - Bacillus subtilis (ATCC 6633) *Candida albicans (ATCC10231), Bacteroïdes 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.

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.

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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 of 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**

MFT acceptance criteria

Industry :

Positives : ≤ 0,1% of MFT units

0 if < 3000 units

Contamination rate = Upper 95% confidence limit x100%
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)

Routine steps *(systematically)*
Team change
Changes in primary packaging (vials, stoppers)
Any change in filling vessels
Sampling process
New manipulation or adjustment during the aseptic process
Environment monitoring and IPC
Changes in the transfer of the filled vials for stoppering, crimping
Stopping and restarting the equipment after an operator intervention during filling process

Simulation elements (industry)

Exceptional process elements
A maintenance intervention
Changing an accessory (filter, gloves, purging the system)
Cleaning intervention
Modification of the environment conditions (overpressure change in the limits)

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Operators Validation

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Goals

- The aseptic operations depends mainly of 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 an 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
- ISO 13408-1 : Aseptic processing of Health care products-Part 1:General Requirements (1998)
- EC Guide to GMP for medicinal products and active pharmaceutical ingredients, annex 1, Rev 1996
- Manufacture of sterile products (2003)
- 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)
- PDA Technical Report N° 36 : Current practice in the validation of aseptic processing (2001)
- Bussières JF, Mise en place d'un protocole de validation microbiologie en hémato-oncologie, Pharmactuel *Vol. 39 N° 4 Août - Septembre 2006*

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