

# **GMP and Monitoring of Environmental biocontamination:**

## ***The priceless contribution of a MALDI Biotyper***

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## → Concerns:

- Pharmaceutical industry / Cosmetology
- Food industry
- Diagnostics tests industry
- ...

→ Includes the **control of microbial contamination** along the whole production process, from raw material to final product.



**The inter-relationship between quality assurance, good manufacturing practice, quality control and in-process control**

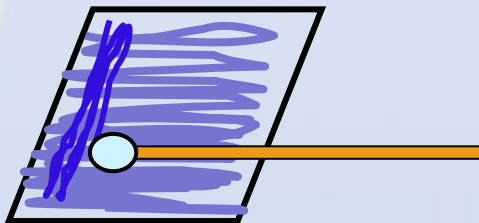
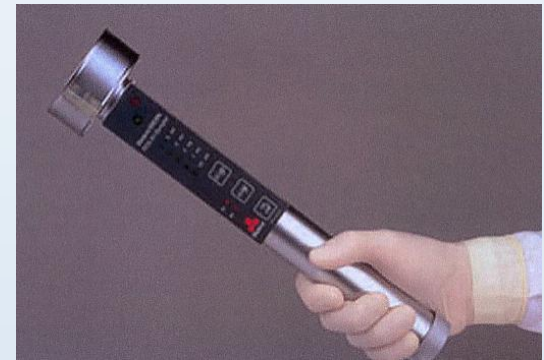
- **Quality control** : part of GMP concerning
  - Sampling, specifications and testing
    - Necessary and relevant tests to perform
  - Organization, documentation, release procedures
  
- **In-process control** : part of GMP concerning
  - Any test on a product, the environment or equipment used during manufacturing process

# Control of microbial contamination during manufacture

- **Environmental cleanliness and hygiene**
  - Sources of contamination of a product
    - Working surfaces, fixtures and equipment
    - Stagnant water
    - Personnel, protective garments
- **Quality of starting material**
  - Raw material
    - Storage conditions
  - Many grades of water
- **Packaging, storage and transport**

- **Architectural design**
  - Separation of each stage of production
  - Classification of rooms according to
    - required degree of microbial and particular cleanliness
    - required air supply and differential of pressure
  - Limited number of persons, limited movement in critical areas
- **Microbiological control of raw materials**
- **Microbiological control of environment**
  - air, surfaces, water

- **Control of airborne contamination**
  - Aspiration
  - Sedimentation
- **Control of surfaces**
  - RODAC plates
  - Swabbing



- Rooms are submitted to various required levels of cleanliness.
- Industry **standards** are defined, for example:
  - ISO 14644-1

Max. Conc. (particles/m<sup>3</sup>) : size ≥0,5 μm

• ISO 1		
• ISO 2	4	
• ISO 3	35	
• ISO 4	352	
• ISO 5	3 520	10 CFU/m <sup>3</sup> air
• ISO 6	35 200	
• ISO 7	352 000	
• ISO 8	3 520 000	100 CFU/m <sup>3</sup> air
• ISO 9	35 200 000	



# Recommended limits for microbial contamination

<b>Grade</b>	<b>Air sample (CFU/m<sup>3</sup>)</b>	<b>Settle plates (CFU/4h)</b>	<b>RODAC plates (CFU/plate)</b>	<b>Glove print (CFU/glove)</b>
<b>A</b>	<b>&lt;1</b>	<b>&lt;1</b>	<b>&lt;1</b>	<b>&lt;1</b>
<b>B</b>	<b>10</b>	<b>5</b>	<b>5</b>	<b>5</b>
<b>C</b>	<b>100</b>	<b>50</b>	<b>25</b>	<b>-</b>
<b>D</b>	<b>200</b>	<b>100</b>	<b>50</b>	<b>-</b>

- Define a **control schedule** for performance of the monitoring samples
- For each room:
  - Define **acceptability and alert thresholds**
  - Define **corrective actions**

## Air/surface samples

- In general, standards requires **only a microorganisms counting**
- Identification is not required

BUT...

**Identification** may be informative to **detect a problem** more rapidly, before the alert threshold is reached

- Production unit 1
- Daily control of
  - 2 surface points
  - 1 air point
- Acceptability threshold for surface controls:  
 $\leq 2$  CFU/plate

Critical points	January 2015 - CFU/plate											
	1	2	3	4	5	6	7	8	9	10	11	12
S1	0	0	1	2	0	1	2	2	2	0	1	1
S2												
A1												

→ OK, no alert

# Example (2)

Critical points	January 2015 - CFU/plate											
	1	2	3	4	5	6	7	8	9	10	11	12
S1	0	0	1	2	0	1	2	1	1	0	2	1
S2												
A1												

Microorg. ID:

*Candida albicans*

*C. alb.*  
+ CNS

*Bacillus*  
sp.

*C. alb.*  
+  
*Bacillus*  
sp.

*C. alb.*

CNS

*C. alb.*+  
*Micrococ-*  
*-cus sp.*

*Bacillus*  
sp.




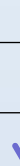

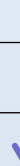
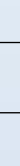


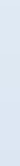
- Recurrence of *Candida albicans*
- *C.alb.*, reservoir = human/animal skin-mucosa  
→ suggest human-borne contamination, probable failing hygiene from a staff member

# Example (3)

- Corrective/preventive action: who were the involved staff members (SM)?

Critical points	January 2015 - CFU/plate											
	1	2	3	4	5	6	7	8	9	10	11	12
S1	0	0	1	2	0	1	2	1	1	0	2	1
S2												
A1												

										
	<i>Candida albicans</i>	<i>C. alb.</i> + CNS	<i>Bacillus sp.</i>	<i>C. alb.</i> + <i>Bacillus sp.</i>	<i>C. alb.</i>	CNS	<i>C. alb.</i> + <i>Micrococcus sp.</i>	<i>Bacillus sp.</i>		
	SM 2	SM 2	SM 1	SM 2	SM 2	SM 1	SM 2	SM 1		

- *C. alb* linked to **SM 2** → Corrective actions:
  - Protection of skin lesions
  - New training

# What do they suggest?

- *Staphylococcus spp*
  - Human-borne contamination
    - Check adequacy of changing facilities and gowning, i.e.
- *Bacillus spp*
  - Environmental contamination
    - Check entry of equipment into cleanroom, i.e.

- **Before MALDI Biotyper:**

- Biochemical methods
  - For non clinical microorganisms:
    - **ID to gender** when possible or
    - **Gram appearance** only
- Frustrating!!

- *Pseudomonas sp.*
- Non fermenting Gram negative bacilli
- *Bacillus sp.*
- B+
- C+
- ...

- **With MALDI Biotyper:**

- Efficient ID of environmental microorganisms
- Possible monitoring of contamination by these  $\mu$ org
- Action plans are more specific and efficient



- 1, Sphingomonas parapaucimobilis, ++, 2.22249083480005
- 2, Sphingomonas pseudosanguinis, +, 1.87024376503824
- 3, psepau, Sphingomonas paucimobilis, +, 1.83687465754116
- 4, Sphingomonas sanguinis, +, 1.77894953404709
- 5, psepau, Sphingomonas paucimobilis, +, 1.71784723534006
- 6, Sphingomonas sanguinis, -, 1.39996763663569
- 7, Sphingomonas yabuuchiae, -, 1.38643478386013
- 8, psespe, Pseudomonas extremorientalis, -, 1.34813786587527
- 9, psepau, Sphingomonas paucimobilis, -, 1.19291842484405
- 10, neimen, Neisseria meningitidis, -, 1.17275990650255

## ***Bacillus sp.***

- 1, Exiguobacterium aurantiacum, ++, 2.27313856836564
- 2, Candida\_sorbosa[ana] -, 1.30206855546366
- 3, Lactobacillus fermentum, -, 1.28610535195844
- 4, Vibrio cholerae, -, 1.25284481101925
- 5, Streptomyces lavendulae, -, 1.25130160993391

- 1, Dermacoccus nishinomiyaensis, +++, 2.34699159530819
- 2, Dermacoccus nishinomiyaensis, ++, 2.21234456189358
- 3, Dermacoccus nishinomiyaensis, ++, 2.19597245695734
- 4, dernis, Dermacoccus nishinomiyaensis, +, 1.83187074187804
- 5, Lactobacillus crispatus, -, 1.40777636813167
- 6, psespe, Pseudomonas brassicacearum, -, 1.31255333250293
- 7, Arthrobacter creatinolyticus, -, 1.30801819401156
- 8, Arthrobacter parietis, -, 1.3052652806702
- 9, Microbacterium liquefaciens, -, 1.28138057509926
- 10, Enterococcus devriesei, -, 1.28055838185214

## MALDI Biotyper ID

*Pseudomonas brenneri*

...

*Sphingomonas parapaucimobilis*  
*Delftia acidovorans*

...

*Paenibacillus glucanolyticus*

...

*Exiguobacterium aurantiacum*  
*Brevibacterium casei*

...

*Kocuria rhizophila*  
*Dermacoccus nishinomiyaensis*

...

...

- >5500 spectra in the last classical database version
    - >2500 different species
    - Wide range of environmental microorganisms
  - Excellent results by direct ID.
- First technology able to allow such performance, excepting sequencing.

- GMP and Monitoring of Environmental biocontamination are required in all accredited production structures.
- Even when alert threshold defined in procedures are not reached, **microorganism ID allow a better traceability of potential problems.**
- **MALDI-TOF MS** is a **rapid and inexpensive** alternative to sequencing for the **ID of environmental microorganisms.**
  - **MALDI Biotyper** has become a technology as useful in **environmental control labs** than in **clinical labs.**