Production of an injectable bacteriophage preparation: identification of the associated critical points

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CONTEXT AND OBJECTIVES

Patient with a **total knee prosthesis** following an accident in 2017: **repeated and recurrent infections**

→ Treatments recommended in the consultation meeting: **antibiotics and prosthesis cleaning**

→ **Collection of joint fluid:** Presence of *P. aeruginosa.* Indication for phagotherapy

→ **Phagogram** (pherecydes) **:** *P. aeruginosa* sensitive to the strains PP1450 and PP1777

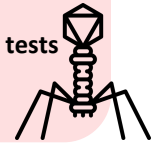
→ **Request from the Traumatology ward:** preparation of intra-articular injections : cocktail of bacteriophages 10^{10} PFU/mL

→ **Design of the circuit** : application to the french health authority (ANSM) for compassionate use

→ **3 injections in the operating room :** 1 per week

Objectives :

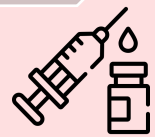
- **To introduce the critical points of the preparation**
- **To analyse the carried out tests**



MATERIAL AND METHODS

1 - Definition of the circuit

Preparation under a type II laminar flow hood (LFH) in a clean room (B class) → Sterilizing filtration (poly ether sulfone filter of 0,22 um) → Syringe filling → Double aseptic packaging under flow → Labeling of the external packaging (asepsis operating room)



In order to reduce the spread of phages :

- completion of the preparation **after all those of the week**
- followed by a **double cleaning** of the LFH with a **disinfectant detergent**
- then the weekly bio-cleaning of the clean room associated with the aerosolization of hydrogen peroxide.

2 - Analysis Identification of critical points for the **preparation**, the **manipulator** and the **environment**

3 - Measures to secure critical points

Sterility test *Bacteriology and hygiene laboratory*	Sample of the **finished preparation and environmental samples** on agar after each preparation under the LFH
Endotoxin test *Control laboratory*	**Limulus test** on the sample of the **finished preparation**. Endotoxin concentration limit calculated according to the European Pharmacopoeia at 0.034 EU/mL
Residual contamination *Bacteriology and hygiene laboratory*	Specific samples by **2 post preparation swabs** (under the field and on the area where the phage vials were placed)
Dangerousness of the preparation	**Multi-professional approach** (bacteriology, hygiene, occupational medicine), information meeting with the pharmaceutical team

RESULTS

Analysis of the 5M diagrams : **17 critical points in total with consequences**

- Non-compliant preparation (sterility defect, endotoxin level too high, phage titer not compliant...)
- Residual contamination of the LFH and/or the clean room (risk of cross-contamination..)
- Contamination of the manipulator

Sterility test	**No CFU counted**
Endotoxin test	C1 = 0,016 EU/mL ; C2 = 0,010 EU/mL et C3 = 0,011 EU/mL Concentrations below to 0,034 EU/mL
Residual contamination	**No lysis range** on *P. aeruginosa* agar
Dangerousness of the preparation	**List 1 of pathogens, waste treated via the classic DASRI channel**

DISCUSSION – CONCLUSION

The critical points of preparation have been identified.

Optimization of the preparation:

- **Different risk analysis (FMECA type)**
- **Calculation of the bacteriophage titer in the finished preparation**

REFERENCES

Phagothérapie en France: une alternative à l'antibiothérapie ; C Méloni *et al.*; Dossier du CNHIM Avril 2021, XLII, 2

Pharmacopée Européenne – Monographie 2.6.14 Essai des endotoxines bactériennes et 5.1.10 Recommandations pour la réalisation de l'essai des endotoxines.

Classification des agents biologiques. Article R. 4421-2 du Code du travail