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Quality Control of Phage APIs

The Belgian Experience

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IABS Webmeeting on Veterinary Use of Phages - November 19-20, 2024

Disclaimer

- Sciensano is a non-for-profit public health institution
- I am a civil servant with no ties to industry
- I am a microbiologist, not a pharmacist, regulator nor jurist
- All opinions expressed here are my own

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The Belgian magistral story started in 2016.

famhp
federal agency for medicines and health products

Unit Scientific and Technical Advice – DG PRE

Federal agency for medicines and health products
Eurostation II - Place Victor Horta 40/40
1060 Brussels
www.afmps.be

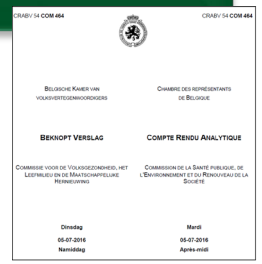
Procedure No :	FAMHP/H/I/15/09/2016/165
Concerning :	Scientific-Technical Advice: "Natural bacteriophages"
Applicant or Legal representative :	Lab MCT – Burn Unit – Military Hospital Queen Astrid

2016

2016

"Set up a framework for magistral phage preparations"

Dr. Maggie De Block, Minister of Public Health



Natural phages can be used as unauthorised raw material in a **magistral preparation** if compliant to provisions of a monograph (Royal Degree 19/12/1997) and upon prescription for an individual patient

The raw material of the active substance needs to be accompagnied with a **certificate of analyses**, coming from a « Approved Belgian laboratory » or OMCL

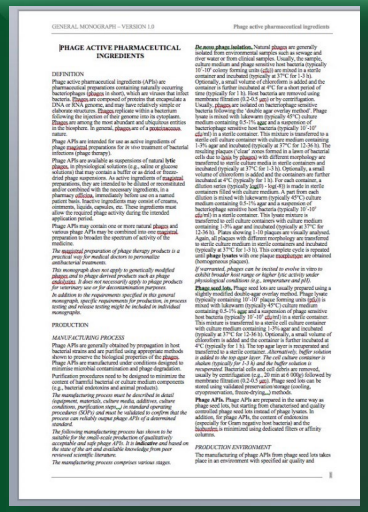
This laboratory is **responsible** to classify the material as conform (or not)

This lab needs to **decide in all independence on which analyses are required** to make this decision

2018

Team Pirnay, hunting for an OMCL lab.

2018



PHAGE ACTIVE PHARMACEUTICAL INGREDIENTS

DEFINITION

PRODUCTION

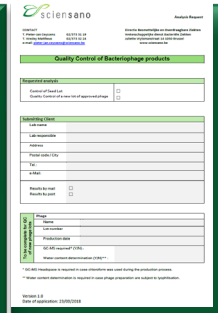
MANUFACTURING PROCESS

PRODUCTION ENVIRONMENT

Phage API monograph published

Pirnay et al., Viruses. 2018;10(2)

2019



Quality Control of Bacteriophage products

Master seed
Production host → Seed lot

MDR strain → Phage Bank (Queen Astrid Military Hospital) → Screen & Produce → Quality Control* (Sciensano) → CoA & prescription → Hospital Pharmacy → Magistral preparation

General European OMCL Network

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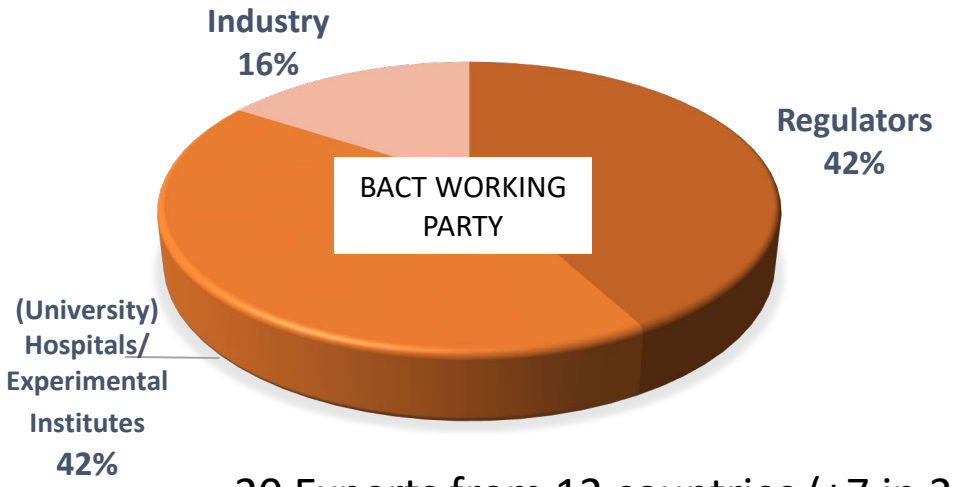
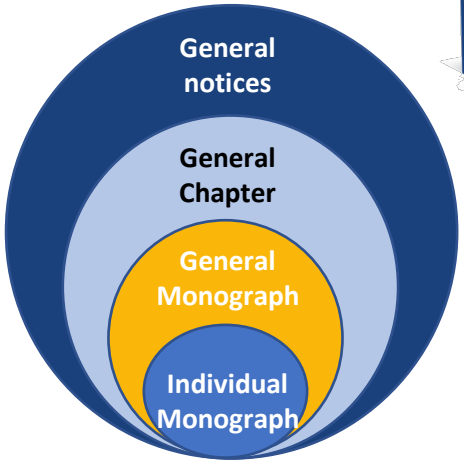


20 Experts from 12 countries (+7 in 2023)

2019 — Belgian request for General Chapter



2021 — "General Chapter on phage APIs is feasible and meaningful "

2023 — Public consultation in Pharmeuropa 35.2

April 2024 — Adopted in 178th Ph. Eur. Commission session and published in Ph.Eur 11.6 (Nov 2024)

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5.31. PHAGE THERAPY MEDICINAL PRODUCTS

This general chapter is published for information.

It offers a framework of requirements for phage therapy active substances and medicinal products for human and veterinary use and their production and control.

The provisions of the chapter do not exclude the use of alternative production and control methods that are acceptable to the competent authority.

1. DEFINITION

Bacteriophages (phages) are viruses that infect bacteria and depend on their bacterial host for replication. Phages consist of a genome comprised of single or double stranded DNA or RNA, encapsulated in a protein capsid.

Phage therapy medicinal products (PTMPs) are preparations of naturally occurring or genetically modified phages used to treat or prevent human or veterinary bacterial infections.

A PTMP can contain one phage, i.e. a single phage therapy active substance, or a mixture of phages, combined with excipients. PTMPs can be administered by various routes and are available in different dosage forms.

2. PRODUCTION

2-1. *GENERAL PROVISIONS*

Phages are obtained by propagation in bacterial host strains and are purified using suitable methods.

The production process yields a PTMP of consistent quality and stability. Appropriate in-process testing is implemented at relevant time points and/or key intermediate stages of the process.

Production of PTMPs is based on a well-characterised bacterial cell bank and phage seed-lot system using a host-phage combination that has been shown suitable. Typically, a two-tiered system is used. Where justified, a single-tiered system may be used.

PTMPs may be prepared on-site as doses for an individual or for a few patients, based on specific clinical needs.

PTMPs require an appropriate framework to ensure the desired quality, hereinafter referred to as the quality system. The extent of the quality system is driven by the risks for the patient concerned, such as microbial contamination. Risk assessment is employed to determine the level of risk and the required level of quality assurance to achieve appropriate product quality.

2-2. *BACTERIAL CELL BANKS*

Bacterial host cells used for PTMP production must be described in detail. This includes information on the source

Microbial purity. The absence of microbial contaminants is determined by plating or any other suitable method.

Viability. The number of viable cells is determined by a plate count or any other suitable viable cell count method.

Phage sensitivity. The susceptibility of the strain to the phage therapy active substance is demonstrated using a plaque assay or any other suitable method.

Absence of detrimental phages. The absence of phage particles that could be detrimental to the quality of PTMPs is confirmed.

If a working cell bank (WCB) is used for production, it is a clonal derivative of the MCB and complies with the requirements for MCB.

2-3. *PHAGE SEED LOTS*

Phage seed lots used in PTMP production are derived from a single phage clone and must be characterised in detail. Information on the phage source, nucleotide sequence and susceptible bacterial species and/or strains is to be provided. Other parameters such as plaque morphology or phage morphology are determined, if relevant.

Phages whose genome contains sequences coding for known or potential detrimental genetic factors, e.g. antibiotic resistance determinants, toxins or lysogeny modules, are avoided, unless otherwise justified and authorised. For genetically or chemically modified phages, the modifications must be described and their effects characterised.

A phage master seed lot complies with the following requirements:

Identification. The phage seed lot is identified by a suitable method.

Microbial purity. The absence of microbial contaminants is demonstrated by a suitable method.

Phage purity. The absence of extrinsic phage contaminants is confirmed by a suitable method; however, as intrinsic phages may be unavoidable when using clinical isolates for production, their presence may in this case be justified and authorised when controlled by a suitable method.

Potency. The infectious phage titre is determined by a plaque assay or any other suitable method.

If a phage working seed lot is used for production, it is a clonal derivative of the phage master seed lot and it complies with the requirements for the phage master seed lot.

2-4. *PRODUCTION AND PURIFICATION*

Production of PTMPs is based on a bacterial cell bank and phage seed-lot system, in which cross-contamination between different phages and bacterial host strains is strictly avoided. The genetic stability of the phage is determined by sequencing a suitable number of purified harvests followed by a comparison against the phage master seed lot sequence.

Raw materials of pharmaceutical grade are used. Raw materials of biological origin also comply with the requirements of general chapter *5.2.12. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products*.

- Natural and modified phages
- Master-seed Production, purification & final lot testing
- Off-the-shelf *and* magistral preparations
- **Diversity of targeted bacteria vs. extreme narrow spectrum of phages**
- Include possibility of adaption to patient isolate

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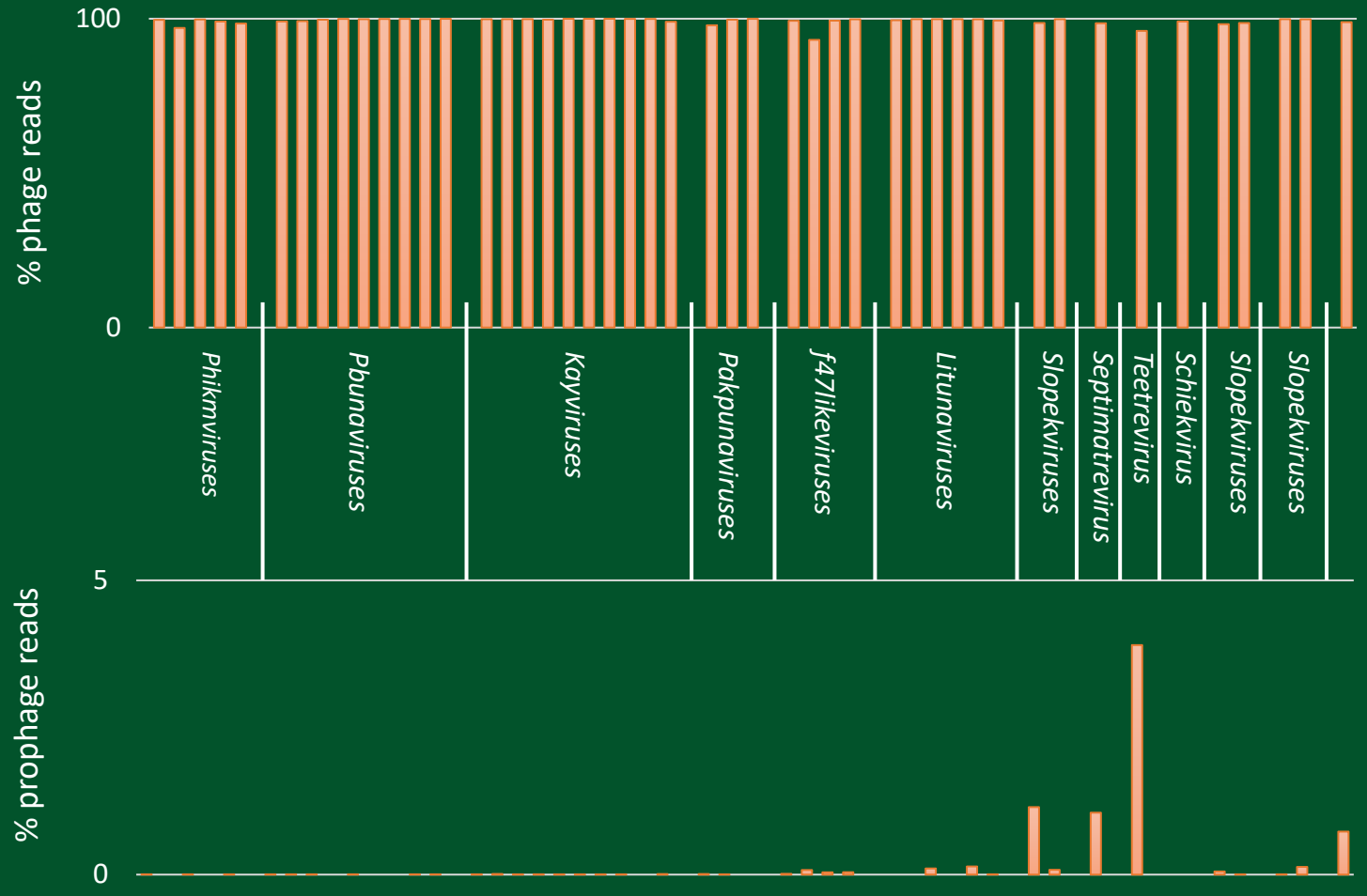
5.31. PHAGE THERAPY MEDICINAL PRODUCTS

Phage purity. The absence of extrinsic phage contaminants is confirmed by a suitable method; however, as intrinsic phages may be unavoidable when using clinical isolates for production, their presence may in this case be justified and authorised when controlled by a suitable method.

The average purity of investigated phage APIs is 99,3%.



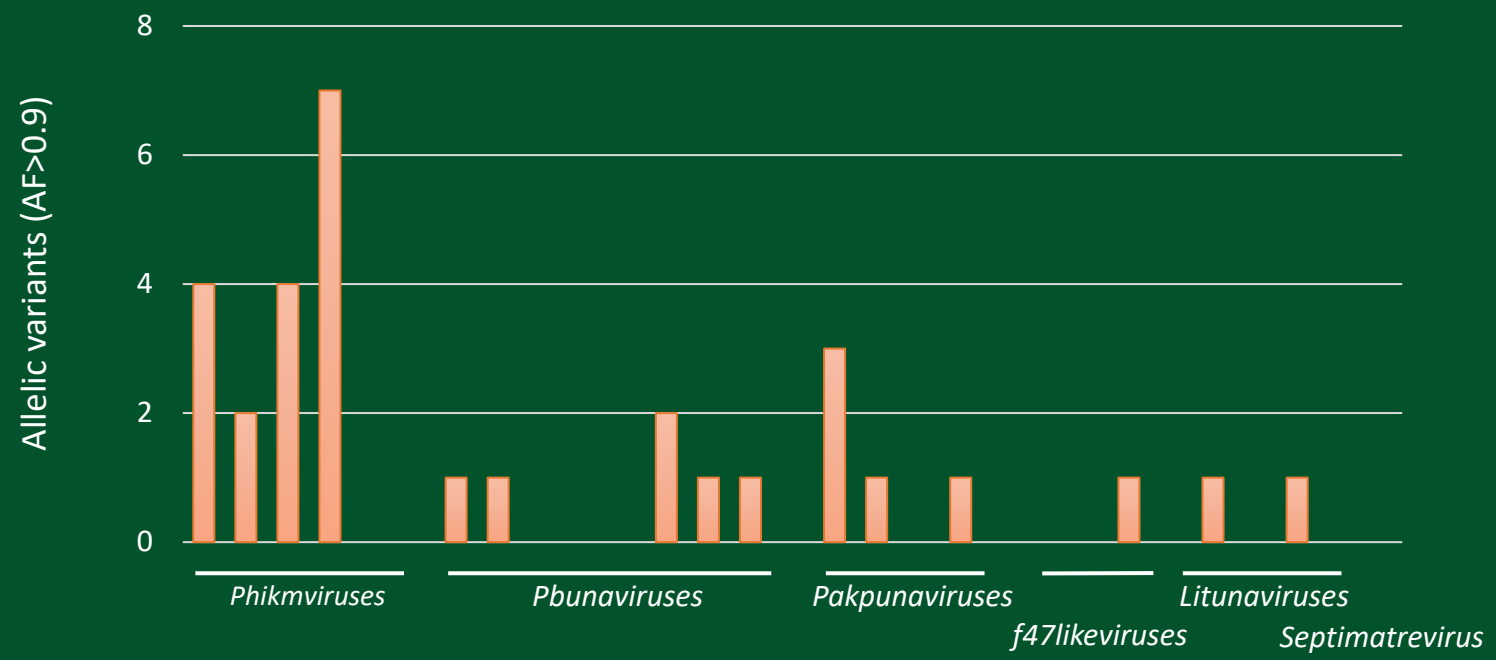
5.31. PHAGE THERAPY MEDICINAL PRODUCTS

2-4. *PRODUCTION AND PURIFICATION*

The genetic stability of the phage is determined by sequencing a suitable number of purified harvests followed by a comparison against the phage master seed lot sequence.

What is ‘genetic stability’ depends on the phage (genus).

Analysis of amount of **majority variants** in comparison to master seed:



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2-4. *PRODUCTION AND PURIFICATION*

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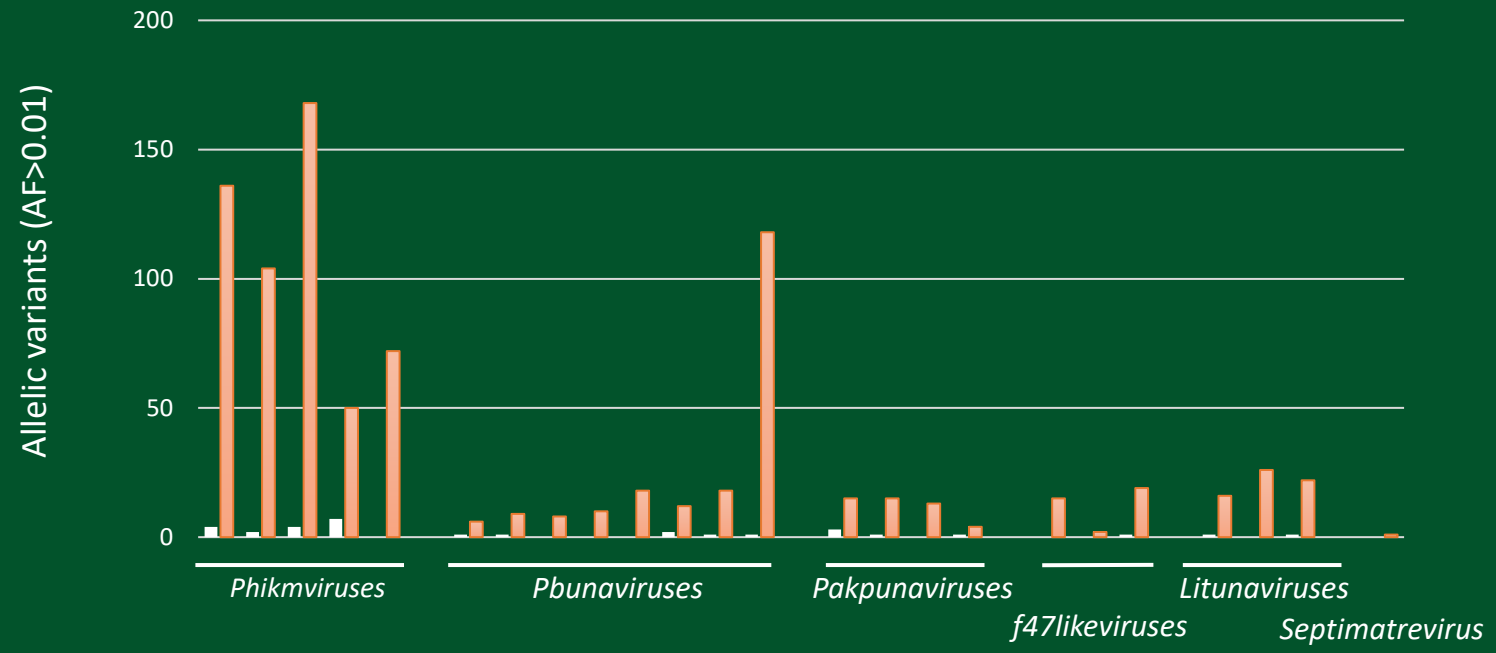
Analysis of amount of **minority variants** in comparison to master seed:



Know and understand the biology of the phage.

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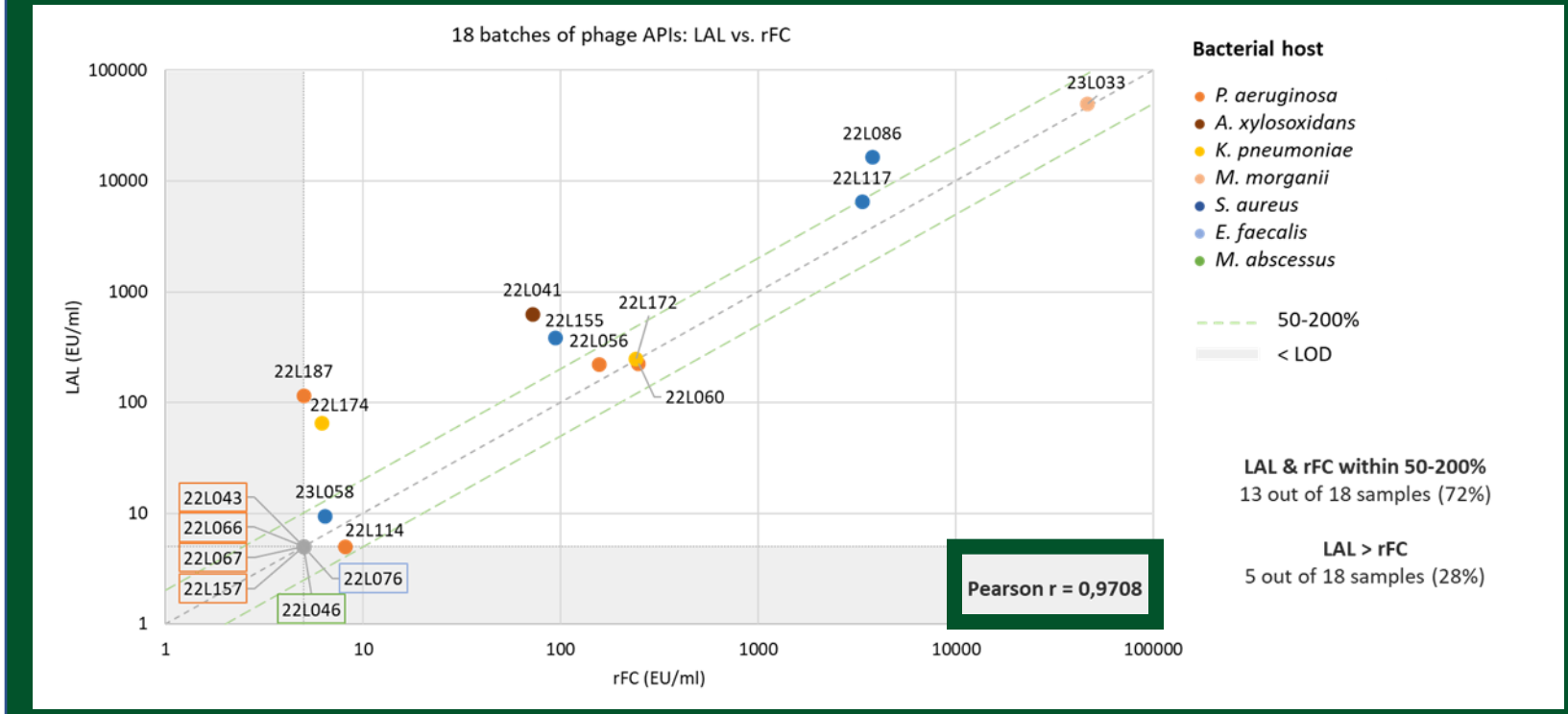
5.31. PHAGE THERAPY MEDICINAL PRODUCTS

2-4. *PRODUCTION AND PURIFICATION*

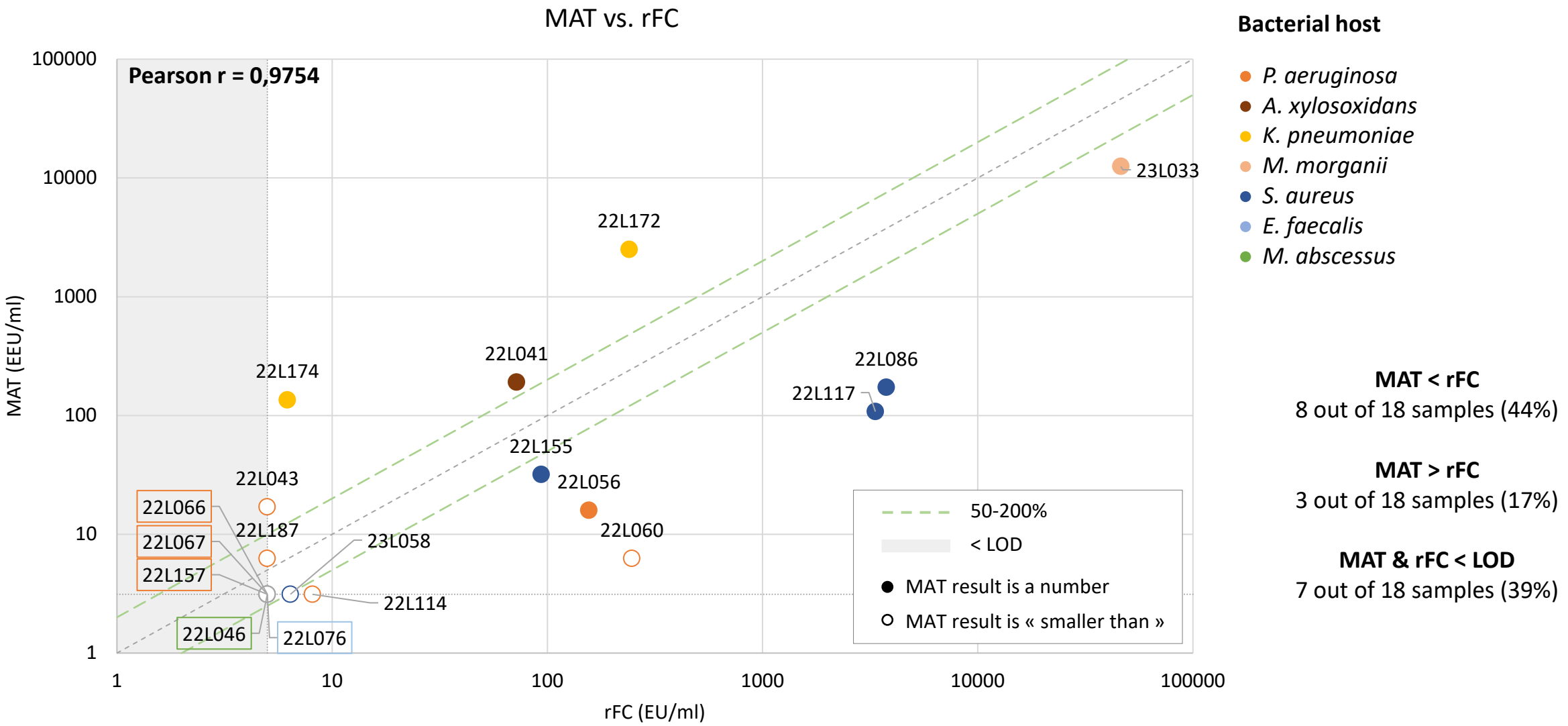
Host-cell impurities and contaminants. Contaminants and other potentially toxic substances derived from the host cells (e.g. endo- and exotoxins, host-cell proteins, host-cell DNA, temperate phages) are absent or within the established specifications.

Recombinant factor C testing performs equally well as LAL in quantifying endotoxins (LPS), with less interference.



… but what about other pyrogens?

MAT results are, surprisingly, comparable or lower than rFC.



Bacterial host

- *P. aeruginosa*
- *A. xylosoxidans*
- *K. pneumoniae*
- *M. morganii*
- *S. aureus*
- *E. faecalis*
- *M. abscessus*

MAT < rFC
8 out of 18 samples (44%)

MAT > rFC
3 out of 18 samples (17%)

MAT & rFC < LOD
7 out of 18 samples (39%)

5.31. PHAGE THERAPY MEDICINAL PRODUCTS

2-5. *FINAL LOT*

Potency. The infectious phage titre of each phage is determined by a plaque assay (expressed in PFU/mL or PFU/mg) or other suitable method and complies with the established specification for the particular preparation.

European Directorate for the Quality of Medicines & HealthCare

BACTERIOPHAGE POTENCY DETERMINATION (2.7.38)

Public consultation in Q2 2025

General remarks

- Quality control of phage APIs needs to deal with **wild variety** of phages and (especially) production hosts
- Tested phage APIs are **genetically very pure (>99%)**, with thresholds of allowed genetic variation might be defined at the genus level
- **Pyrogenicity** of phage APIs for human use is **generally low**, although only IL-6 is tested and intrinsic anti-inflammatory response of phages might interfere
- The **lack of reference material and standardized tests** (potency, genetic testing) is problematic for wide implementation

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European Directorate for the Quality of Medicines & HealthCare

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Funding :

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