EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Case studies on the Guideline on quality, safety and efficacy of veterinary medicinal products specifically designed for phage therapy

Focus group meeting

Case Studies

11 May 2023

Disclaimer & Objective

Disclaimer

- ✓ These slides have only been provided for exemplification of this draft guideline in public consultation.

Objective:

- ✓ The objective of this presentation is to spark discussion on the guideline

Case study 3

Initial marketing application for a parental phage product, with flexible quantitative and qualitative composition and delivered to the user upon the determination of the adequate combination of phages.

Case study 3 - Introduction

- Initial marketing authorisation of a multiphage VMP (external phage product).
- Final product with flexible composition of phages from a phage library (composed by natural phages, obtained from dogs with external otitis).
- The adequate combination of phages is delivered to the user after being determined by the MAH.
- Indication: Treatment of external otitis caused by *Pseudomonas aeruginosa*
- Route of administration: ear administration, once per day during 3 consecutive days.
- Target species: dogs
- Host manufacturing bacteria: qualified *Pseudomonas aeruginosa* strain + (when necessary) training on the strain isolated from the dog to be treated

Case study 3: Bacteriophages isolation and characterization

- ✓ Identification
- ✓ *In vitro* characterization
- ✓ Growth Properties

Details in Guideline section 4.2
Quality documentation:

IIIa.2A3. Characterization
IIIa.2C. Production and control of starting materials
IIIa.2E. Control tests on the finished product

Pseudomonas aeruginosa : 30
Pseudomonas non-*aeruginosa*: 15
Bacteria from the ear flora: 25

	Pseudomonas aeruginsa phage			
Phages characteristics	PH1	PH2	…	PH12
Source species	Dog 1	Human 2		raw sewage
Isolation date				
Isolation strain				
Plaque size				
Plaque Morph				
Plate stock (PFU/ml)				
CsCl purified (PFU/ml)				
EM morphology				
Pseudolysogeny Assay	Pass	Pass		Pass
Sequence				
Accession N°				
ORF				
tRNA				
Toxin/Virulence genes	None	None		None
Lysogeny cassettes	None	None		None
Abx-Resistance genes	None	None		None
Closest relative				
Genus				
Growth properties				
spectra				
30 Clinical Pseudomonas aeruginosa				
EOP>0.1	X/30	X/30		X/30
EOP>0.001	X/30	X/30		X/30
15 Pseudomas spp				
EOP>0.1	X/15	X/15		X/15
EOP>0.001	X/15	X/15		X/15
25 Ear flora strains				
EOP>0.1	X/25	X/25		X/25
EOP>0.001	X/25	X/25		X/25

Case study 3: Bacteriophages isolation and characterization (2)

Composition of parental product:

- Initial candidates for multi-phage product:

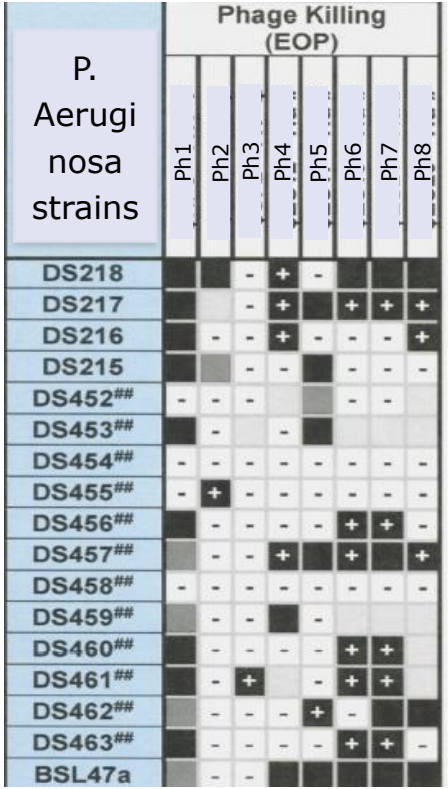
Ph1, Ph2, Ph3, Ph4, Ph5, Ph6, Ph7, Ph8.

While Ph3 has a narrow spectrum range it is considered justified based the uniqueness of its bacterial receptor

- The MAH defined that the final product will be composed by the 3 phages with the highest EOP in a phagogram with the bacterial strain from the dog to be treated

~~representative preparation~~ not needed

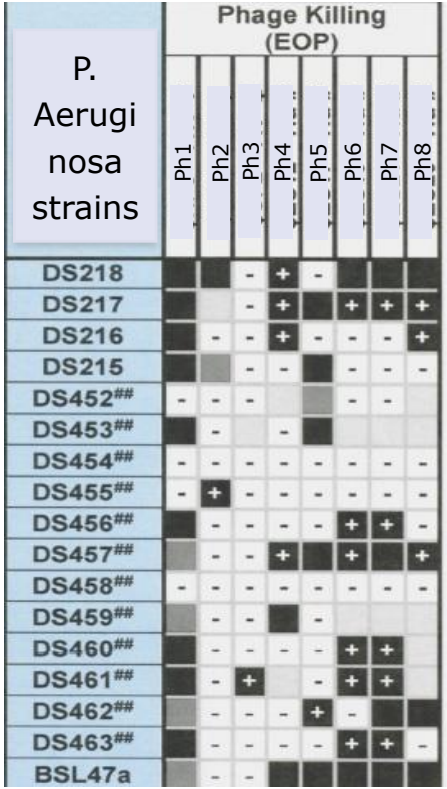
Details in guideline section 4.2 Quality documentation:
IIIa.2A1.Qualitative and quantitative composition
IIIa.2A2. Product development



Case study 3: Bacteriophages isolation and characterization (3)

Qualification of each propagating strains:

- No toxin or resistance genes detected in the final bulk of each bacteriophage strains



Details in guideline section 4.2 Quality documentation:
IIIa.2A1.Qualitative and quantitative composition
IIIa.2A2. Product development

Case study 3: Quality package

IIIa.2A1. Qualitative and quantitative composition

✓Qualitative composition: Description of all different bacteriophage strains which may be employed : PH1 to PH12

✓Quantitative composition:

- Maximum number of bacteriophages: 3 bacteriophages (decided by the MAH)
- The titre of each bacteriophage in PFU/ml

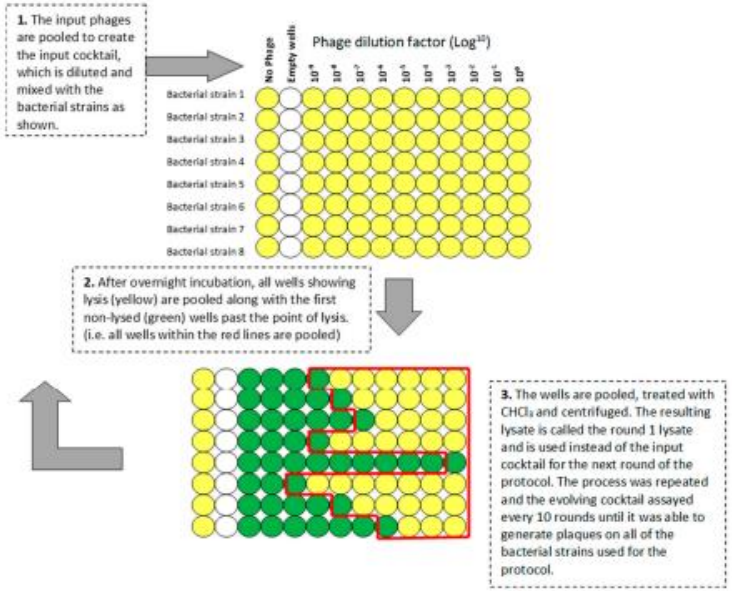
Details in guideline section 4.2 Quality documentation (IIIa.2A1. Qualitative and quantitative composition and IIIa.2A2 Product development), and section 5 Post marketing authorisation changes

Case study 3: Manufacturing process

- adapted GMP rules about the use and the monitoring of premises (taking into account the number and the size of manufactured batches)
- A batch is the 3 doses necessary for one treatment
- Each batch is obtained further to the blending of 3 bulks from the phages with the highest EOP (and at least > 0,1) in a phagogram of the bacterial strain from the dog to be treated
- The batch release parameters:
  - ✓ Phage characterisation
  - ✓ PFU/ml (determined in the dose/determination study – most often ~10^7)
  - ✓ Endotoxin < clinically qualified threshold
  - ✓ ….

Case study 3:
Manufacturing process – training on the dog's bacterial isolate

When no phage of the bank has an EOP>0.1 in the phagogram, the 3 phages with the highest EOP are trained on the isolate.
[the risk of the potential transfer of bacterial antibiotic resistance and toxin genes is justifed as negligible because the product is administered to the animal which already harbours high amounts of the training strain]



Case study 3: Safety package

IIIa.3A2. Pharmacology

Summary of the available information on PK/PD (PK results drawn from the study with an experimental otitis model).

Details in guideline sections 4.3 Safety documentation (IIIa.3a2. Pharmacology

Case study 3: Safety package

IIIa.3A3. Toxicology

✓No specific toxicology studies submitted (single-dose toxicity, repeated dose toxicity, reproduction and developmental toxicity).

✓**Risk assessment on safety identified the following risk**: comensal flora could be modified by these bacteriophages. Risk analysis based on results drawn from phage characterization (25 strains from the ear flora) and from the efficacy studies (pre-clinical and clinical).

✓The standard battery of **genotoxicity test** is omitted as it has been appropriately justified by bibliography - bacteriophages do not interact with DNA, or other chromosomal material of eukaryotic cells.

✓**Carcinogenicity studies** are omitted, as no theoretical concerns based on literature, in silico, and/or in vitro study results, or due to a mode of action have been identified.

Details in guideline sections 4.3 Safety documentation (IIIa.3A3. Toxicology)

Case study 3: Safety package

IIIa.3A4.1. Special studies

✓**Immunogenicity and immunotoxicity:** No specific studies provided because the product is administered externally

✓**Development of resistance and related risk in humans:** An analysis of the risks of developing/spreading resistances in treated animal, in the environment and the related risk to the users is provided.

IIIa.3A45. User Safety

Qualitative risk assessment and corresponding warnings added to the SPC.

IIIa.3A6. Environmental risk assessment

Risk assessment provided. The product is considered safe for the environment.

IIIa.3B. Residue

Not applicable for dogs

Details in guideline sections 4.3 Safety documentation (IIIa.3A4.1 Special studies, IIIa.3A5, User safety and IIIa.3A6 Environmental risk assessment)

Case study 3: Efficacy package

IIIa.4A1. Pharmacology

- ✓ Mechanism of action:
  - Lysis of *P. aeruginosa in vitro* (Quality part)
- ✓ Host range: Host range established *in vitro* for each single bacteriophage (Quality part)
- ✓ Posology.
- ✓ Comparability data to support variations to include new bacteriophages in the bank

IIIa.4A2. Development of phage resistance and related risk in animals

A reflection is provided upon the risk of developing/spreading resistances target animals.

Details in guideline section 4.4 Efficacy (IIIa.4A1. Pharmacology and IIIa.4A2. Development of phage resistance and related risk in animals)

Case study 3: Efficacy package

IIIa.4A3. Dose determination and confirmation studies

Dose determination study

✓Experimental model: dogs with otitis further administration of different strains of with *P. aeruginosa*

✓Groups of treatment: each dog challenges with 1 *P. aeruginosa* strain: 1 ear is treated with the product and the other with saline (control ear)

✓Treatment: the products is administered once a day during 3 consecutive days with 3 different dilutions of the product (3 groups).

✓Efficacy parameters: clinical and bacterial cure

✓Safety & pharmacokinetic parameters: phagogram of *P. aeruginosa* strains & phage concentration in the earwax at the end of the treatment ; clinical monitoring of the dog (particularly skin and mucosae of the head)

Details in guideline section 4.4 Efficacy (IIIa.4A3. Dose determination and confirmation studies)

Case study 3: Efficacy package

IIIa.4A4. Tolerance in the target animal species

✓**Objective:** To address the identified safety risk: whether comensal flora could be infected by these bacteriophages.

✓**Target species:** Healthy dogs

✓**Treatment:** The batches used for the dose determination study were administered to healthy dogs.

✓**Groups of treatment:**

-Cross-over study where dogs were compared to themselves (flora sequenced before and after treatment) and were successively administered 2 batches

✓**Study parameters:** Comparison of the composition of the comensal flora before and after treatment, and for each cross-over group.

✓**Results and conclusion:** The representative preparation does not significantly disturb the comensal flora in dog ear.

Details in guideline section 4.4 Efficacy (IIIa.4A4. Tolerance in the target animal species)

Case study 3: Efficacy package

4.4.2. Clinical trials

- ✓ **Objective:** To assess the efficacy of the preparation.
- ✓ **Target species:** dogs with *P. aeruginosa* chronic otitis
- ✓ **Study design:** 2 groups: treated & control (saline)
- ✓ **Treatment:** according to the SPC (further a phagogram).
- ✓ **Study parameters:** Clinical & bacterial cure.

Details in guideline section 4.4 Efficacy (4.4.2. Clinical trials)

Case study 3: SPC, Leaflet and Labelling

- **SPC and LEAFLET**

  External preparation

- **LABELLING**

  3 bacteriophages selected following a phagogram with the bacterial strain from the dog to be treated or trained with it

Details in guideline section 4.1 Administrative information

Case study 3: SPC

1. NAME OF THE VETERINARY MEDICINAL PRODUCT

Phage ear drops, suspension for dogs.

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

1 ml contains:

Active substances *:
Phage against *Pseudomonas aeruginosa* (PHX) ***.....≥ strain specific pass level (PFU/m) **
(*) maximum of 3 different phages
(***) PHX_T : Phage X trained against the isolated strain

Strain-specific pass levels	
PHX	PFU/ml (**)
PH1	> 8.6 log10 PFU/ml
PH2	> 9.5 log10 PFU/ml
...	
PH12	> 9.3 log10 PFU/ml

Excipients:

Qualitative composition of excipients and other constituents:
Excipient 1

The type of strain(s) (3 strains at most) included in the final product will be selected based on the phagogram at the time of manufacturing and will be stated on the label.

Case study 3: Risk Management Plan (post-authorization measures

- **mandatory**
- **Design**

  All batches are delivered with a questionnary the vet should fill in after the completion of the treatment which reviews safety and efficacy parameters of the treatment

Case study 4

Post-marketing update of the product of case 1, to update the product in accordance to new bacterial strains which have emerged in the field.

Thank you for your attention

Any questions