Case study on a VMP with flexible composition to treat diarrhea caused by ETEC in post-weaning piglets

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Please note

- This case study is purely hypothetical, and examples including descriptions of assays, parameters or studies are non-binding.

- The case study is only intended to provide a high-level example of the principles described in the guideline.

Target bacteria

- ETEC produce
  - fimbriae → bind to the enterocytes
  - toxins → after binding, toxins are released

→ Post weaning diahrrea

- ETEC strains in Europe

Fimbriae	Toxins
F4	STa
F18	STb
F5	LT
F6	Mixes
F41	--

The most common pathotype is F4, STb, LT.

Luppi, A., Gibellini, M., Gin, T. *et al.* Prevalence of virulence factors in enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhoea in Europe. *Porc Health Manag* **2,** 20 (2016). https://doi.org/10.1186/s40813-016-0039-9

General characteristics of the bacteriophage medicinal product

- Flexible composition from a parenteral preparation composed by well characterised natural phages, obtained from sewage of a pig farm.
- Indication: Treatment of diahrrea caused by enterotoxigenic *E. coli* (ETEC).
- Route of administration: Oral administration during 7 consecutive days.
- Target species: Post-weaning piglets.

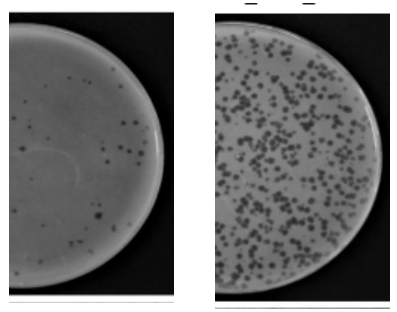
Bacteriophages isolation and characterisation (i)

Properties of *E. coli* phages				
Phage characteristics				
Source Location	Seawage of pigs farms			
Isolation Date	2020/03/01	2021/04/25	2022/10/01	2022/02/10
Isolation strain	EC-1	EC-2	EC-3	EC-4
Plaque morphology and plaque size	Clear, 0.5 mm	Clear, 0.5 mm	Clear, 1-2 mm	Clear, 3 mm
Plaque stock (PFU/ml)	$3.00x10^9$	$4.20x10^9$	$7.40x10^8$	$6.20X10^8$
Electronic microscopy morphology				

Table adapted from: Gibson SB et al, Constructing and Characterizing Bacteriophage Libraries for Phage Therapy of Human Infections. Front Microbiol. 2019 Nov 12;10:2537. doi: 10.3389/fmicb.2019.02537

Bacteriophages isolation and characterisation (ii)

Phages growth in vitro using the manufacuting bacteria *E. coli* MG1655

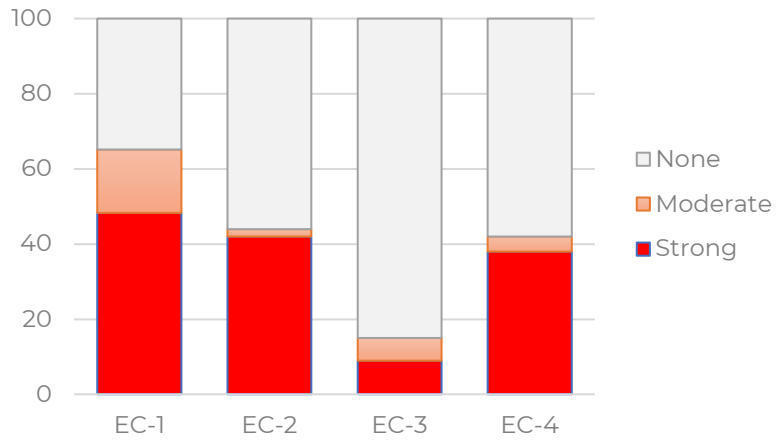


Properties of E. coli phages

	Sequence			
Isolation strain	EC-1	EC-2	EC-3	EC-4
Accesion No.	KY608976	KY608977	KY608978	KY608979
Genome (pb)	168.188	170.254	166.373	169.782
Toxin/virulence genes, lysogenic cassettes, Ab-resistance genes	None	None	None	None

Table adapted from: Gibson SB et al, Constructing and Characterizing Bacteriophage Libraries for Phage Therapy of Human Infections. Front Microbiol. 2019 Nov 12;10:2537. doi: 10.3389/fmicb.2019.02537

Phage killing phenotypes of E. coli tested strains



Graph adapted from: Gibson SB et al, Constructing and Characterizing Bacteriophage Libraries for Phage Therapy of Human Infections. Front Microbiol. 2019 Nov 12;10:2537. doi: 10.3389/fmicb.2019.02537

ETEC strains tested for charaterisation: 89

Bacteriophages isolation and characterisation (iii)

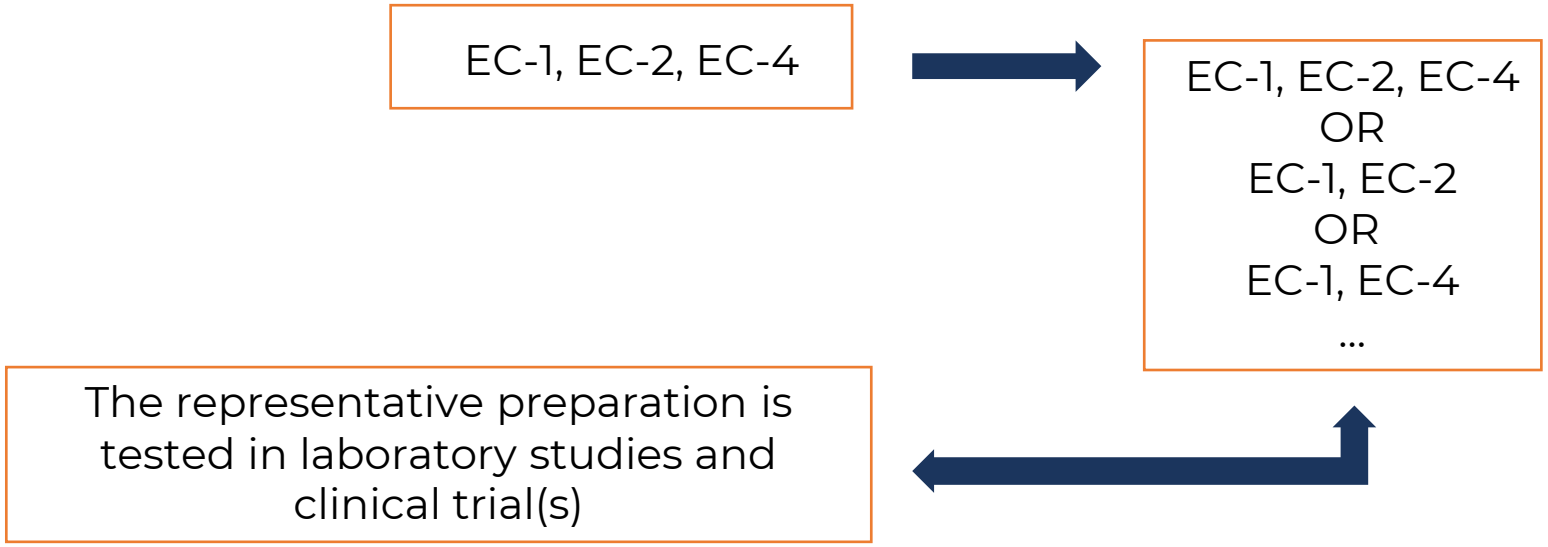
Composition of parental product:

✓ Initial candidates for multi-phage product:

EC-1, EC-2, EC-3, EC-4

However, EC-3 is not considered justified based e.g on shown host range+grow properties by the MAH.

✓ The MAH defined the parental product as composed by:



Is my product safe?

Based on a risk-profile study (scientific publications, previous experience, in vitro data, in vivo studies (laboratory studies and clinical trials)).

Safety for the target species

Risk assessment on safety identified the next risk: comensal flora could be affected by these bacteriophages (risk addressed in the **TAS study (Part 4 Efficacy).**

Laboratory study(ies): Tolerance in the target animal species

- ✓**Objective:** To address the identified safety risk identified: comensal flora could be infected by these bacteriophages.
- ✓**Target species:** Healthy piglets at post-weaning
- ✓**Treatment:** Representative preparation administered orally, once per day during 7 consecutive days.
- ✓**Study parameters:** Comparison on the composition of the comensal flora before and after treatment, and between treated and control groups.
- ✓**Results and conclusion:** The representative preparation does not significantly disturb the comensal flora in post-weaning piglets.

Is my product safe?

Safety for the user

Following GL EMA/CVMP/543/03-Rev.1 (hazard identification and characterisation, exposure assessment and risk assessment) no risks were identified.

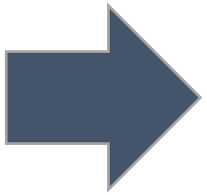
Safety for the environment

It was concluded that no impact to soil bacteria and soil function is associated with the use of the product

Safety for the consumer

MRL assessed prior to the MAA in food producing animals

The establishment of maximal residue limits (MRL), as set out in Commission regulation (EU) 470/2009 for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin



EMA should be consulted for the need of a MRL evaluation

Is my product efficacious “in vivo”?

From in vitro to in vivo efficacy

Pharmacology

- **Mechanism of action**

✓ Lysis of ETEC *in vitro* where all bacteriophages are tested *in vitro* in several ETEC strains (Quality part).

✓ Host range: Host range established *in vitro* per each single bacteriophage.

✓ Posology?

✓ Immune response (repeated administration)?

The representative preparation is tested in laboratory studies and clinical trial(s)

Comparability data to support a flexible composition of monophage or multiphage preparations

Based on **representative/validated *in vitro* or *in vivo* data or parameters,** or based on a scientific justification.

Comparable biodistribution, immune clearance and MOI to demonstrate comparability between representative and alternative preparations.

- **Development of phage resistance and related risk in animals**

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

Is my product efficacious “in vivo”?

Laboratory study(ies) for dose determination and dose confirmation

✓Medicinal product: representative preparation (at different doses)

✓Target species: post-weaning piglets

✓Groups of treatment: control group vs treated group(s)

✓Animals are challenged with an ETEC *E. coli* F4, STb, LT before treatment.

✓Study Parameters:

- **Clinical signs** related to the disease.
- **Shedding in faeces** of ETEC challenge strain.
- **Immune response** against bacteriophages.

✓Results and Conclusions:

- The higher dose of the representative preparation reduced the clinical signs of the disease, and the shedding of tested ETEC strains. No immune response against bacteriophages was detected

Is my product efficacious “in vivo”?

Clinical trial

- ✓**Objective:** To assess the efficacy of the representative preparation.
- ✓**Target species:** Post-weaning piglets affected by ETEC diahrrea
- ✓**Treatment:** Representative preparation administered orally, once per day during 7 consecutive days.
- ✓**Study parameters:** Clinical signs and shedding of ETEC.
- ✓**Results and conclusion:** The parental preparation showed to be efficacious to treat ETEC post-weaning diahrrea by reducing the clinical signs of the disease and the shedding of the ETEC. No safety concerns addressed along the study.

Administrative information, SPC, Leaflet and Labelling

- **SPC and LEAFLET**

  Parental preparation

  EC-1, EC-2, EC-4

- **LABELLING**

  Actual bacteriophages selected in the final product

  EC-1, EC-2, EC-4
  OR
  EC-1, EC-2
  OR
  EC-1, EC-4
  ...

Two years after....

- Bacterial resistance has now developed to EC-2 in the field.
- The MAH wishes to update the marketed product with a trained version of EC-2 which overcomes the bacterial resistance:

EC-2→ EC-2*

Is the trained version of phage EC-2 comparable to the original phage EC-2*?

They have highly similar quality attributes, and no adverse impact on the safety or efficacy of the product is expected (ICH Q5E).

Required quality, safety and efficacy documentation for product update

Description of post-authorisation phage product update	Category of phage product update	Level of changes to manufacturing process(es)	Likely quality data requirements for approval of updated phage product	Likely safety data requirements for approval of updated phage product	Likely efficacy data requirements for approval of updated phage product
Addition of a monophage component which is comparable to a component which is authorised with the marketing authorisation application	# Simplest	# Not substantial	§ Minimal	If monophage components are comparable, safety studies are not expected to be required (post-authorisation changes expected to be approvable based on quality data alone). In the same line, user and environmental risk assessment is not expected to be required. It is advised to consult the Agency for the need of a MRL status.	If monophage components are comparable, target animal safety studies are not expected to be required (post-authorisation changes expected to be approvable based on quality data alone).
Addition of one or more new monophage components to product which are not comparable to a component which is authorised with the marketing authorisation application	$ Complex	$ May be substantial	Unless it can be scientifically justified that the proposed product update does not carry with it unacceptable risks to quality, safety, efficacy and traceability of product, re-validation of manufacturing processes and associated analytical technologies, as well as documentation of comparability between parental and updated product, may be required.	If the impurity profile of the product is not substantially worsened, safety studies might not be required. In high level of complexity or lack of alternative evidence, safety data may be required. New user risk assessment and environmental risk assessment might be needed. It is advised to consult the Agency for the need of an MRL.	If the new monophage components are not comparable to monophage components already present in the product, data from laboratory efficacy studies in target animal species or representative species may be required. In worst case scenarios (high level of complexity or lack of alternative evidence), data from new clinical trials in target animal species may be required. To avoid the requirement for a full efficacy package, alternative tools should be established. For example, it is expected that for *in vivo* studies, surrogate efficacy endpoints established and validated for the parental product might be used.

SPC, Leaflet and Labelling for updated product

- **SPC and LEAFLET**

  Parental preparation

  EC-1, EC-2*, EC-3 ←

- **LABELLING**

  Actual bacteriophages in the final product

EC-2*:
Version of ΦEC-2
which has been trained
to overcome bacterial
resistance

Take-home messages

- A **thorough characterization** of the bacteriophages in a representative sample of target bacteria strains is recommended.

- **Obtaining data to support the comparability** in compounds with flexible composition is of importance, and it should be considered from the beginning of the product development.

- **Risk profiling methodology** to identify inherent risks to the specific product will help to decide the studies needed to support the quality, safety and efficacy of the phage therapy product.

Take-home messages

- **Product updates should be considered from the very beginning** of the development of the parental product.

- The elaboration of plans and protocols for anticipated product updates, formalised in **post-approval change management protocols (PACMP) (see EMA/CHMP/CVMP/QWP/586330/2010),** foster the authorization of these products.

Thank you!