Development of a new diagnostic device allowing a personalized phage therapy

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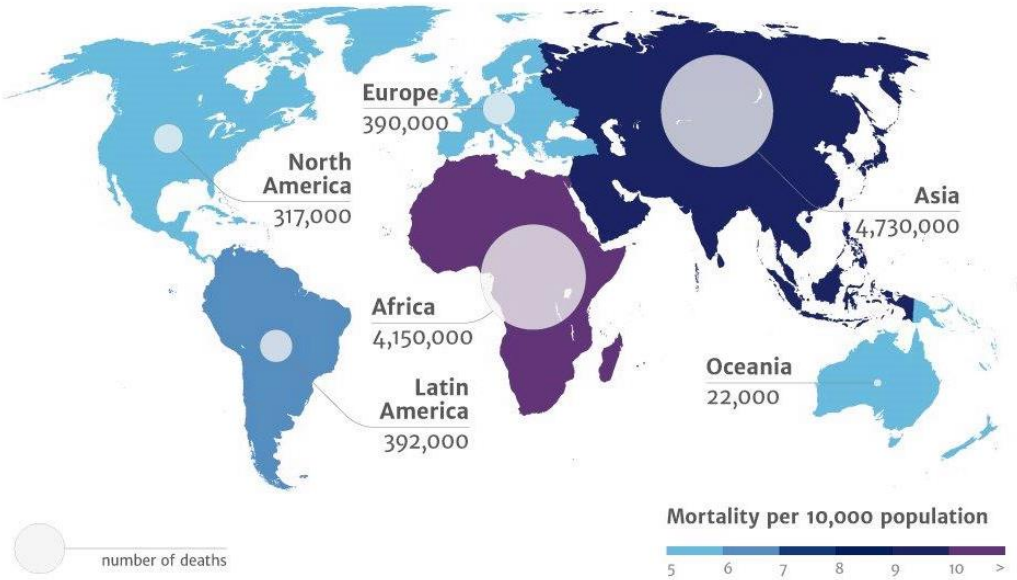
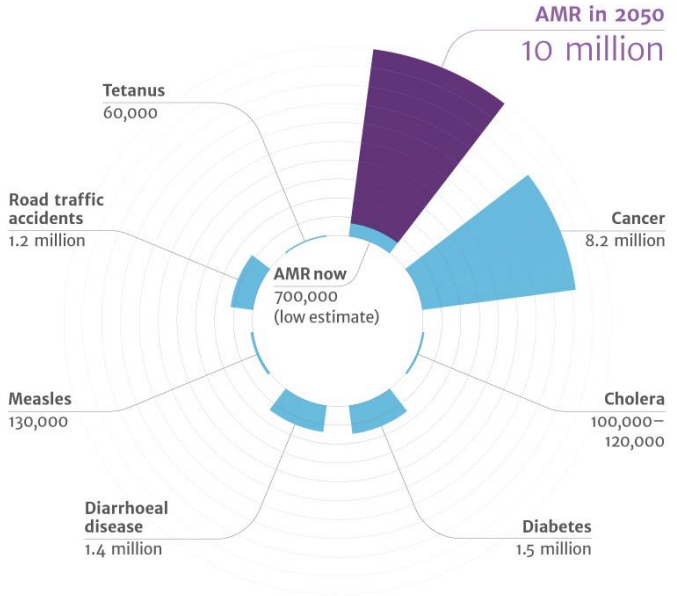
1 June 2022

Impact of Antimicrobial resistance

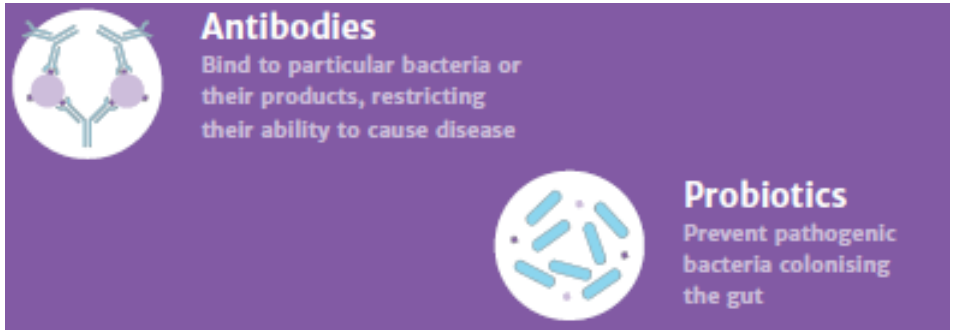
Longer Illeness → Increased Mortality → loss of protection for patients → Prolonged stays in hospital → Increased costs





Source: http://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance -- https://amr-review.org/infographics.html

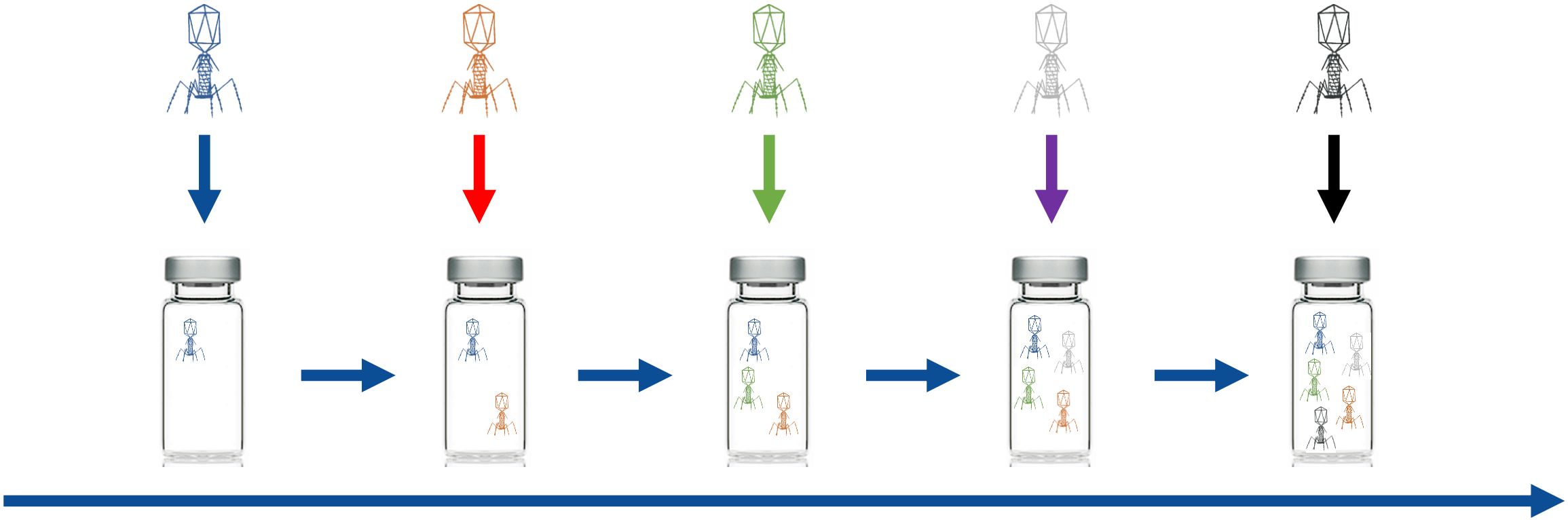
Alternative products to tackle infections



Phage therapy - History

- In August 1915, ten infantrymen in the French army had contracted severe haemorrhagic dysentery
- Investigation of this outbreak was assigned to a young French-Canadian scientist Felix d'Herelle working at the Institut Pasteur
- d'Herelle saw evidence of invisible viruses, which were killing the bacteria
- In 1915, the English bacteriologist Frederick Twort had made similar observations, but due to the war his grant from the Local Government Board came to an end…
- In 1917, d'Herelle published his findings in the proceedings of the French Academy of Sciences and coined the name "bacteriophage"
- In 1919, d'Herelle applies bacterial viruses in therapy (dysentery)

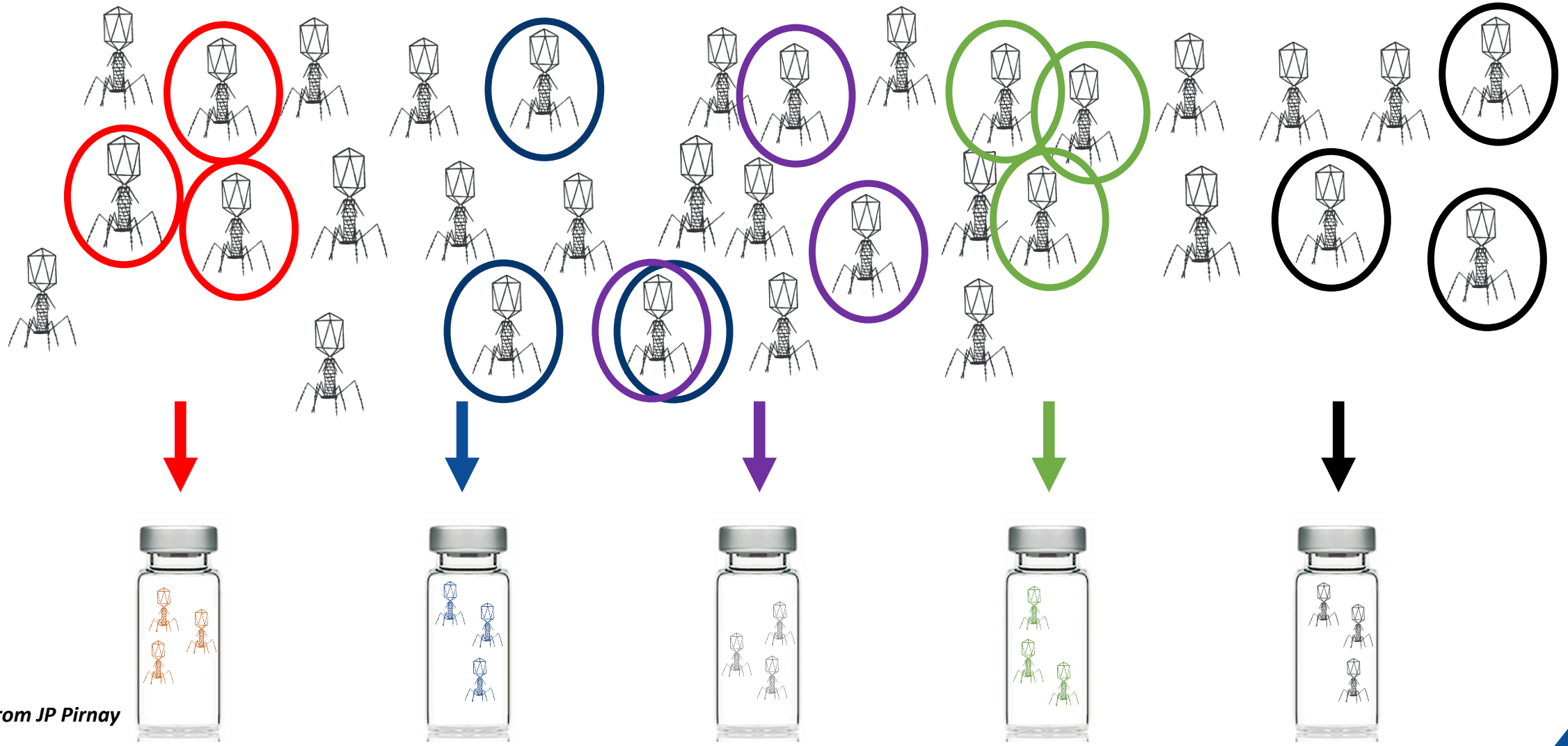
Phage therapy – From mono-phage to cocktail development



Adapted from JP Pirnay

80 years of development but…
What about the duration of the treatment and the emergence of resistance?

The Belgian Model



Adapted from JP Pirnay

A pragmatic solution for phage therapy in Belgium

On 26 October 2016, it was formally agreed that natural phages can be processed by a pharmacist as active ingredients in magistral preparations, providing compliance to a number of logical provisions

Magistral preparations?

- In European and Belgian law, a magistral preparation is defined as “any medicinal product prepared in a pharmacy in accordance with a medical prescription for an individual patient” (Article 3 of Directive 2001/83).

- In general, active pharmaceutical ingredients (APIs) of magistral preparations must conform to the provisions of a monograph in an official pharmacopoeia.

- In addition, non-authorized ingredients may also be used, providing that they conform to the provisions of an internal monograph and are accompanied by a certificate of analysis issued by a Belgian Approved Laboratory.

Phage therapy – The Belgian Model

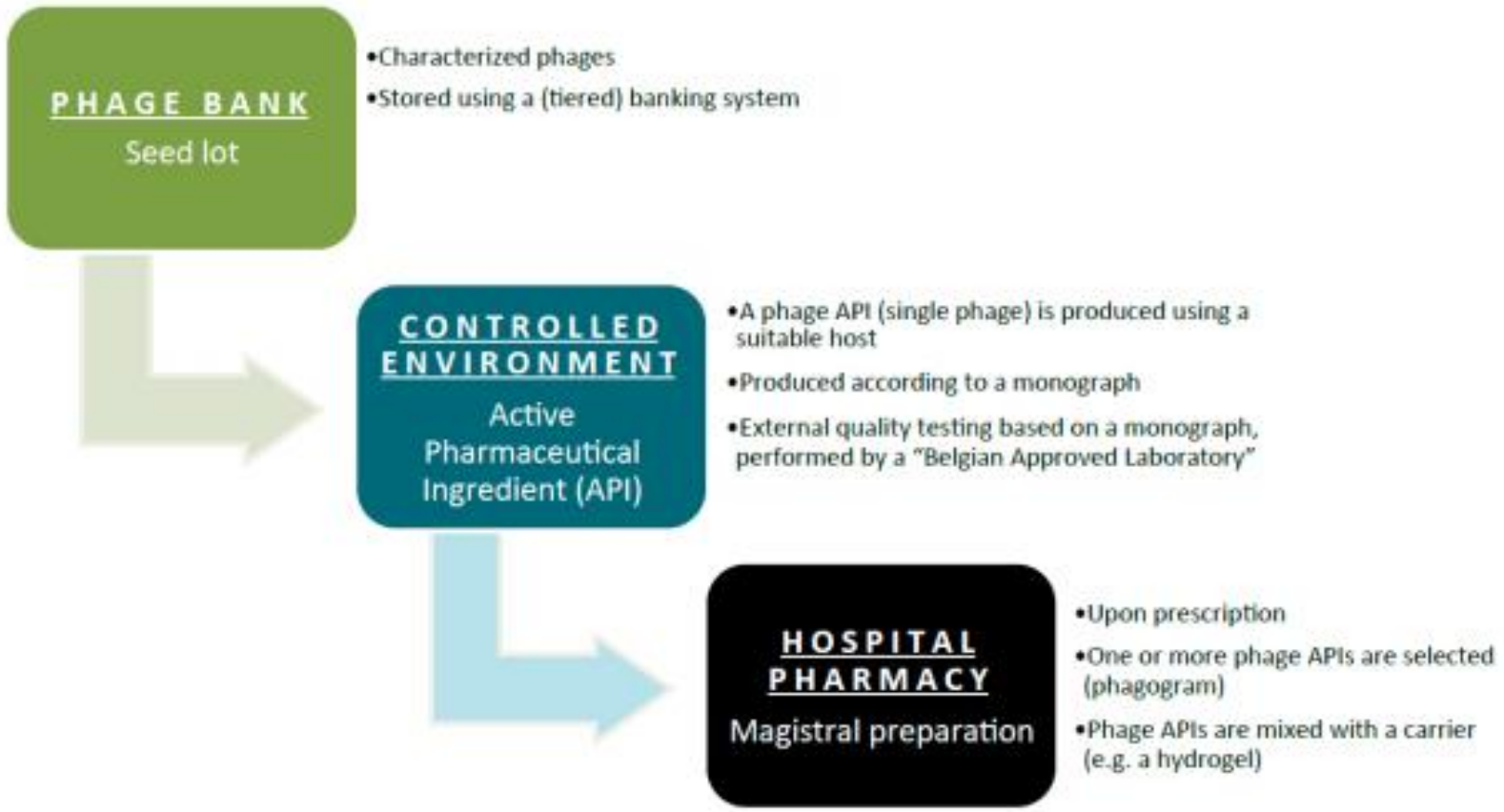


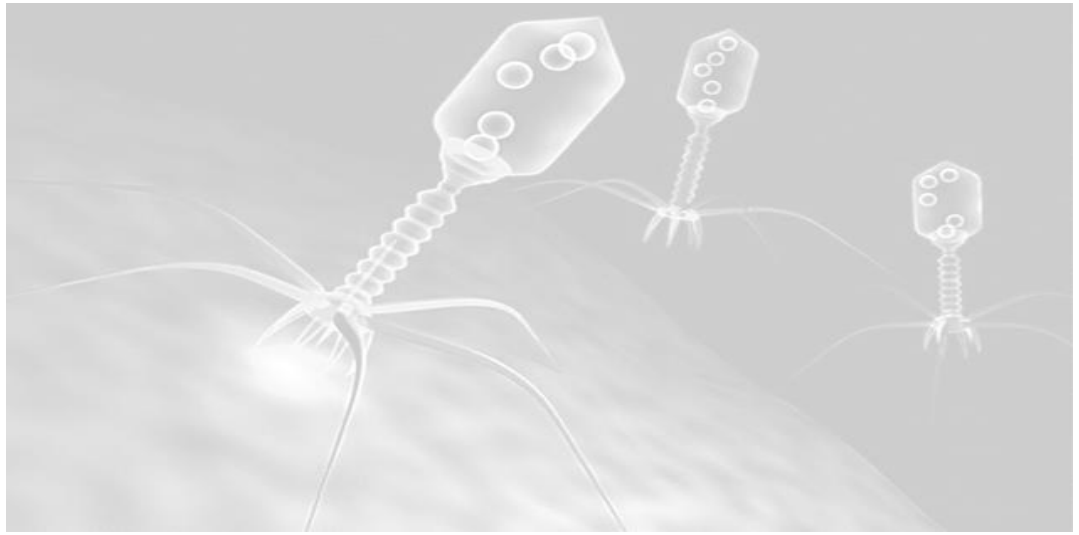
Figure 1. General flowchart of the magistral phage medicine process.

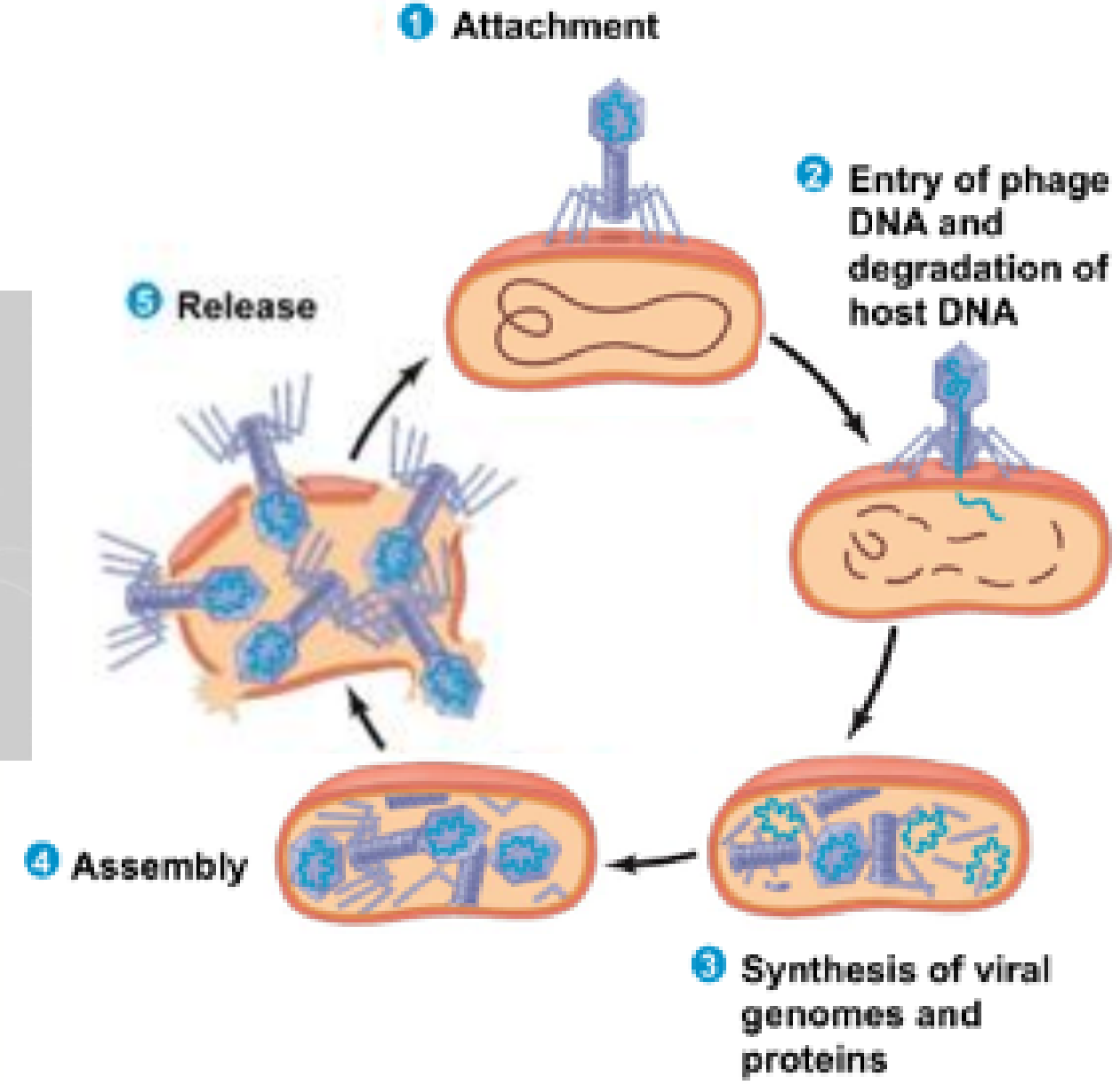
Phage therapy – Pro and Cons

- Pro
  - Bactericidal agents
  - Auto “dosing”
  - Low inherent toxicity
  - Minimal disruption of normal flora
  - Lack of cross-resistance with antibiotics
  - Rapid discovery & evolution
- Cons
  - Not all phages make for good therapeutics.
  - Formulation and application versatility
  - Unclear relationship to the innate and adaptive immune system
  - The challenge of narrow host range.
  - What about biofilm?

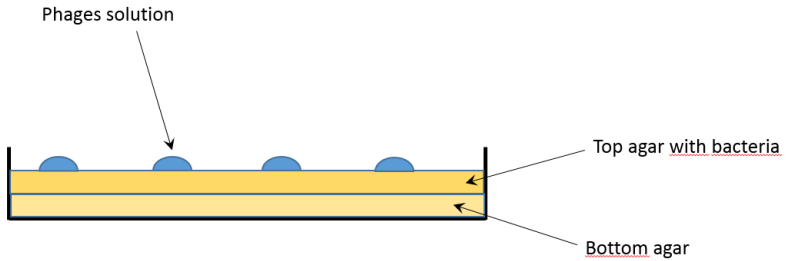
LHUB-ULB has the ambition to support clinical researchs on Phage therapy as a potential tool to treat and/or prevent AM-Resistant infections in Belgium

Phage therapy – How it works

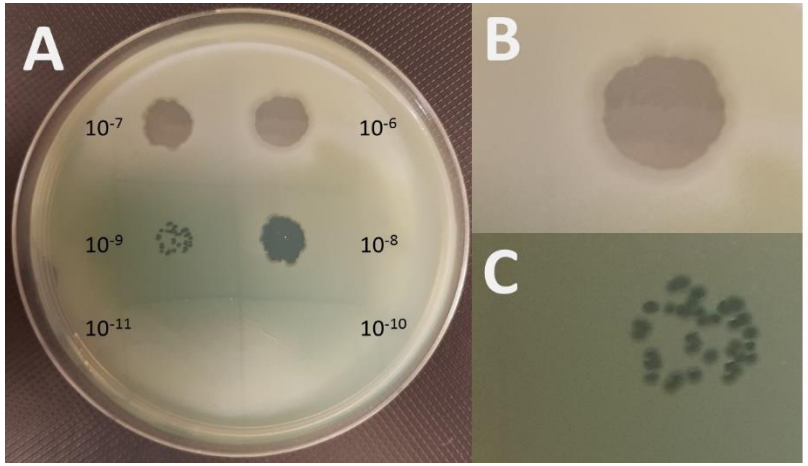




Reference methods for phage susceptibility testing

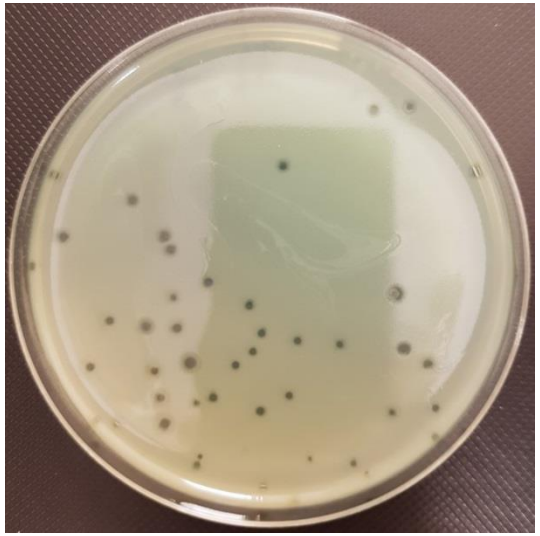


Spot test: distribution of phages solution and bacteria within or on top of agar matrices



Spot test: *Pseudomonas aeruginosa* and phage PEV2 diluted from 10^{-6} to 10^{-11}. (B) Clear zone (dilution 10^{-6}). (C) Plaques (dilution 10^{-9})

Double agar overlay plaque assay: distribution of bacteria into agar layers



Double agar overlay plaque assay with *Pseudomonas aeruginosa and phage PEV2*. Holes are termed plaques. The titration of phage is determined by counting the number of plaques, reported to the dilution, and expressed in PFU/mL (in this case, 41 plaques at dilution 10^{-9} = 4.1 x 10^{10} PFU/mL)

LABORATORY REQUIREMENT FOR A NEW METHOD

- To provide an easy access to phage therapy, new methods need to be developed and require at least some of the following specifications:
  - user friendliness
  - feasibility in every, including small, laboratory hospital
  - Not requiring expensive disposables or equipment
  - Not requiring specialized academic labour
  - Not requiring bulky equipment
  - Fast; i.e. low turn-around time and definitely not more than time needed for AST
  - high sensitivity and specificity
  - standardization of results

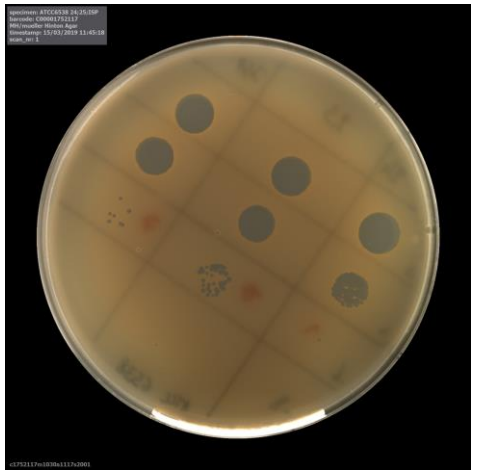
LABORATORY REQUIREMENT FOR A NEW METHOD

- Standardized results must be clear and, as it currently is the case for antibiotics with the “sensitive, intermediate, resistant” classification, results must be easily understood by clinicians to be translated in clinical standardized actions towards patients.

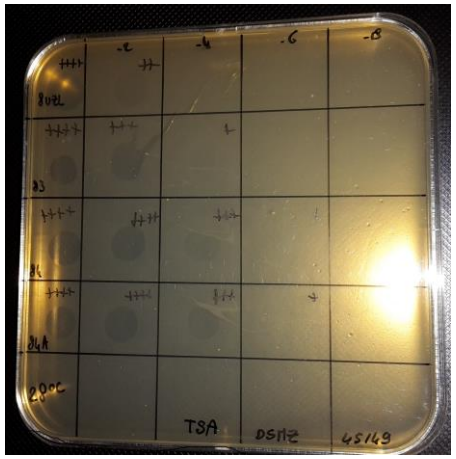
- Clarity and easiness of PST results understanding by clinicians is the cornerstone for a broad use of the phage therapy. Based on the two reference tests and the phage characteristics, a three-level classifications could be suggested, as follows:
  - Insensitive: phage has no activity on tested bacterium
  - Kill and/or inactivate: phage is lethal to bacterium and can be used as passive therapy: Spot test
  - Kill, inactivate, and propagate: phage is lethal to bacteria and can proliferate, and can be used as active therapy: Double agar overlay

In-Vitro Evaluation of Bacteriophages' Activity

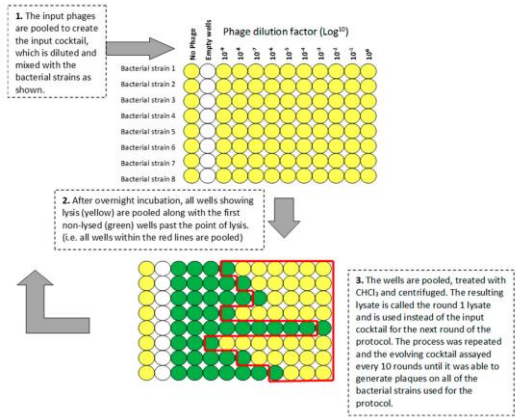
Double agar overlay method

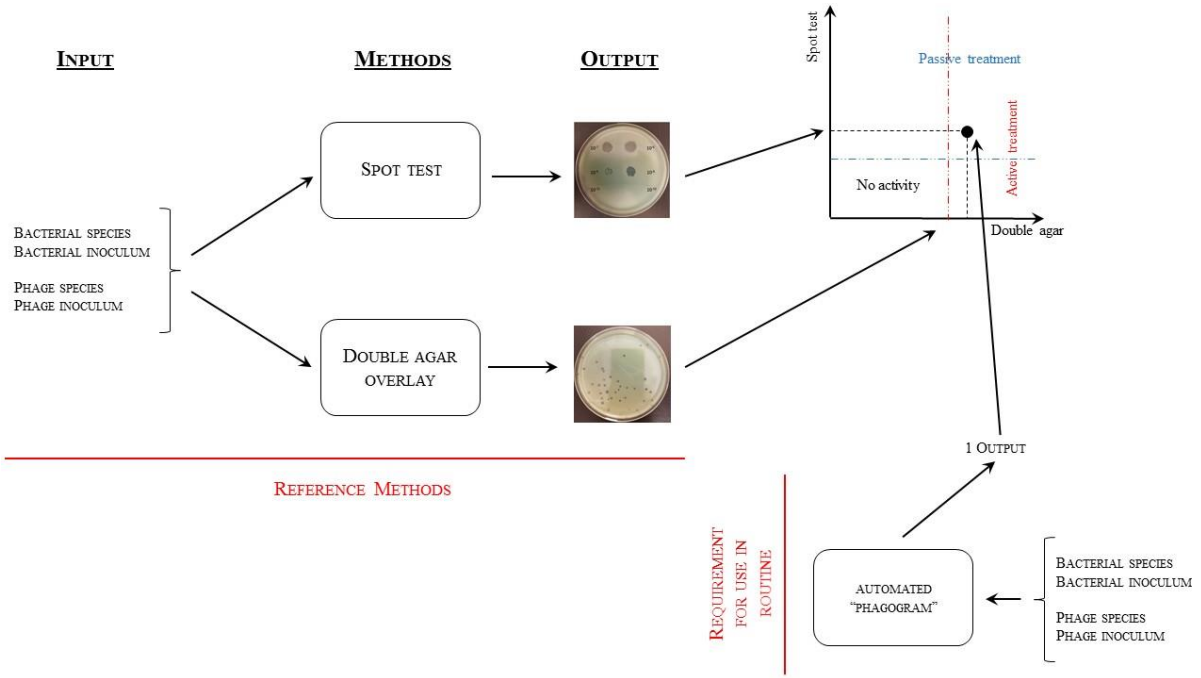


Spot Test



Appelman's method





Organisational structure of CE-IVD certification on Belgian level

EUROPEAN LEVEL

BELGIAN LEVEL

5/4/2017
IVDR
entry into force

21/7/2017
Royal decree
FAMHP

IVDR WG in
Commission
of clinical
biology

IVDR WG
starts formal
meetings with

Call for experts
EU Expert Panel
Joint Research Centre

- Euro Commission (DG SANTE & MDCG)
- TF-ERA (EFLM)
- BioMed Alliance

Joint opinion of RBSLM & IVDR WG
for postponement of IVDR >> EFLM

(c) Devices intended for detecting the presence of an infectious agent, if there is a significant risk that an erroneous result would cause death or severe disability to the individual, foetus or embryo being tested, or to the individual's offspring

RATIONALE

Rule 3c applies to devices intended for detecting the presence of an infectious agent (either the agent itself or component thereof) e.g. bacterial, viral, fungal, parasitic, protozoal infectious agents.
Devices intended for the detection of antibodies against the infectious agent are not covered by this rule.

This rule does not have any specimen type restrictions and is applicable to specimens being tested from the individual, foetus or embryo.

This rule applies if there is a significant risk that an erroneous result would cause death or severe disability. It is the risk of death or severe disability to an individual that must be considered. In this context, the risk of death or severe disability to the individual should take into account that an erroneous result in a healthy individual does not carry the same risk as an erroneous result in (for example) a pregnant, immunocompromised, or vulnerable individual. This rule also applies to an embryo or foetus being tested, or the individual's offspring where an infectious agent can be detrimental to the viability/development of the embryo/foetus leading to death or disability, both current and future e.g. developmental disability

EXAMPLES (non-exhaustive)

Devices intended for detecting the presence of:

- Bacterial pathogens: *Treponema pallidum*, *Chlamydia trachomatis*, *Haemophilus influenzae* type B meningitis, *Neisseria meningitidis*, *Listeria* meningitis (*Listeria monocytogenes*), *Mycobacterium leprae*, *Mycobacterium* spp., *Legionella* spp., *Streptococcus agalactiae*, methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-resistant Enterobacteriaceae (MRE).
- Parasitic pathogens: *Toxoplasma gondii*.
- Viral pathogens: Herpes simplex virus 1&2, cytomegalovirus, Rubella, Measles, Poliomyelitis, Parvovirus B19, Zika.

Acknowledgments

King Baudouin Foundation and Funds Iris-Research Win2Wal fund from the Walloon region

Molecular and Cellular Technology, Queen Astrid Military Hospital, Brussels, Belgium

The Belgian private company, Vésale Pharma, which support our project by providing the phages