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Supplementary information

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# A consensus-based guideline for personalized bacteriophage therapy

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# A Consensus-based Guideline for Personalized Bacteriophage Therapy

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# A Consensus-based Guideline for Personalized Bacteriophage Therapy

# Supplementary explanations

# Supplementary explanations

## Supplementary explanation 1: Recommendations on general principles of personalized bacteriophage therapy

*Background R2-1:* **Phage therapy may be considered for patients suffering from bacterial infections that might be treatable with phages, who qualify for an individual treatment attempt. This implies that standard guideline-based therapy has failed, is unavailable or is not applicable. Moreover, phage therapy may be considered within the context of a clinical trial.**

The decision in favor of phage therapy should be carefully considered on a case-by-case basis, taking into account the specific circumstances and clinical conditions of each individual patient. [18] At present, phage therapy outside of clinical trials mostly follows the legal framework of the individual healing attempt. This refers to patients for whom standard of care therapy has failed or who are not eligible for standard therapy due to toxicity, interactions or other factors that preclude and/or limit its use. Previous areas of administration include, for example, infections caused by antibiotic-resistant bacteria and recalcitrant bacterial infections such as those associated with implants. [21,40-44] In such cases, phage therapy could offer a targeted and effective approach.

Defining treatment success in phage therapy remains one of the most nuanced and currently unresolved challenges in the field. The available data are not yet sufficient to support a definitive, universally applicable definition. In most clinical scenarios, clinical improvement represents a more realistic and patient-centred endpoint than microbiological eradication, although eradication may be an appropriate and achievable goal in selected cases depending on the individual clinical context. Systematic retrospective analysis of documented cases through a registry framework will be essential to generate the data needed to refine and standardize success criteria in the future.

Clinical trials with phages are challenging due to most phages' high specificity, requiring a tailored phage-bacteria match for each patient. This individualized approach complicates standardization and large-scale trial designs in RCTs. Additionally, the biologically dynamic nature of bacteria-phage interactions and potential to develop phage resistance can necessitate therapy adjustments of the investigational medicinal product(s) (IMPs) during the trial. Moreover, the limited shelf-life of these biological preparations may represent a hurdle. Despite these difficulties, clinical trials are essential for establishing robust evidence of the safety and efficacy of phage therapy. Some clinical trials, mostly phase I-II, have been performed or are underway, indicating a favorable safety profile for phage administrations. [24-30] As an example illustrating both the challenges and the importance outlined above, the German Phage4Cure trial [148] can be cited, which focuses on three phages administered via inhalation for the treatment of *Pseudomonas aeruginosa* infections in patients with bronchiectasis. The originally planned funding period, from 2017 to 2020, was extended until 2024 to accommodate the logistical and regulatory preparations required for GMP-compliant manufacturing in Germany, with clinical phase I/II having commenced in 2024. [111] In summary, it cannot be envisioned that conducting clinical trials for all phages and treatment indications might be feasible.

*Background R2-2:* **An interdisciplinary discussion between treating physicians, microbiologists, and infectious diseases specialists, as well as experts involved in phage selection, manufacturing, susceptibility testing, and treatment should precede phage therapy.**

Phage therapy is a highly targeted intervention that requires precise pathogen identification and careful matching of phages to the infecting strain. Its success depends on accurate pathogen identification, phage susceptibility testing, and the careful selection and preparation of appropriate phage preparations. Because no single discipline encompasses all required expertise, coordinated input from treating physicians, microbiologists, infectious diseases specialists, and phage experts is essential. Such interdisciplinary discussion, which may, for example, be referred to as a phage therapy board, ensures that phage choice, manufacturing quality, and therapeutic strategy are aligned, thereby supporting the safe and effective use of phage therapy.

*Background R2-3:* **There is limited data on the safety of using phages in infants, children, pregnant women, breastfeeding women, patients with co-medications, allergies, or immunocompromised patients. However, there is no data suggesting safety issues in these populations. Thus, treatment should be based on an individual risk-benefit assessment.**

In general, phage therapy is considered safe, if quality standards are met. [17,18,20,149] However, the current basis of evidence concerning phage treatment in distinct patient groups is limited. While there seems to be no data pointing at safety concerns, further data are needed for a conclusive evaluation.

Age <18, pregnant and breastfeeding women, infants, and children: While the risks of phage therapy in these groups are presumed to be low, the available data remain very limited. Several indirect observations suggest the potential safety of phage therapy in such cases. For example, preterm infant guts naturally harbor phages. [74] Phages have also been identified in breast milk, where their presence may play a physiological role, potentially supporting healthy child development. [72,73] There is a possibility that therapeutically administered phages could be excreted into breast milk when used by breastfeeding women. [72,73] Data on the placental passage of phages are scarce as well, with only a few *in vivo* studies available. [69-71] Some phage administrations involving infants and children suggest favorable safety profiles for phage therapy. [21,28,60-68]

Comedication: There are currently no known restrictions on the use of phage therapeutics alongside other medications, although the available data on potential interactions are limited. [18]

Role of immunosuppression: One review of 2008 concludes that phage therapy is safe in immunocompromised patients. [59] More recent reviews suggest that phage therapy is safe in underlying diseases or conditions causing immunosuppression, e.g., diabetes, after organ transplantations (kidney, heart, lung), or cystic fibrosis. [20,55-58] There is evidence that phages could have immunomodulatory properties. [45-54] Although the clinical relevance of these immunomodulatory effects remains currently unknown, increased monitoring in respective patient populations may be appropriate.

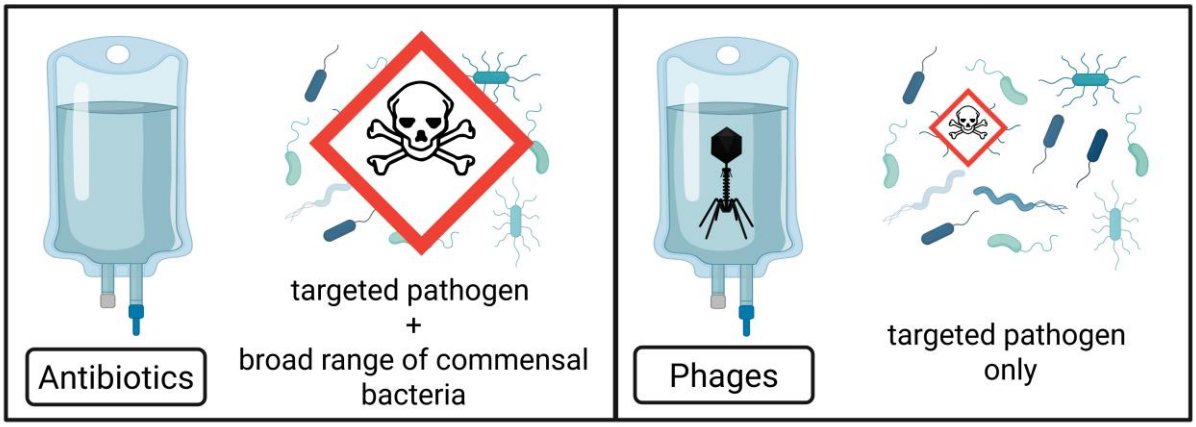
Allergies: Phage therapy involves the administration of foreign proteins, which could potentially cause allergic or anaphylactic reactions, especially with repeated treatments, but no reports of allergic reactions directly attributable to phage therapy have been identified in the available clinical data to the best of our knowledge. [18,19] For instance, eosinophil counts remained within the normal range in certain cases treated with phages, as noted by Międzybrodzki et al. [57] However, emerging evidence suggests that phages may open new avenues for use in modulating immune responses and potentially treating allergic disorders. In 1991, promising outcomes were claimed by Sakandelidze et al., who reported success in over 1,300 patients with "infectious allergoses". [76] Positive results have also been documented in Ukrainian patients with allergies to antibiotics. [75] Notably, a recent case involving a boy with Netherton syndrome and atopic diathesis demonstrated significant improvement, including reduced skin involvement, without any allergic reactions to the phage preparation. [150] In summary, while the available

data on allergic reactions to phage therapy is limited, monitoring for such reactions remains advisable. No allergic reactions directly linked to phage administrations have been reported, and emerging evidence suggests that phages may hold potential for immune modulation in the treatment of allergic disorders.

Spread to patient's contacts: Currently, there is no evidence suggesting clinically significant spread of therapeutic phages to a patient's environment or close contacts. However, this possibility cannot be entirely excluded, particularly in relation to nebulization, excretion in stool, or presence in breast milk.

*Background R2-4:* **The identification of the target bacteria for phage therapy should be culture-based and include species identification and antimicrobial susceptibility testing.**

The key advantage of phage therapy lies in the high specificity of phages: they target specific bacterial species and even strains within a species. However, this specificity also creates challenges, as it requires careful selection of the right phage for each patient. [18] The specificity of phages can be so pronounced that a phage may be effective for one patient but not for another, even if both suffer from bacteria of the same species and capsule type, such as *Klebsiella pneumoniae* [151,152] or *Acinetobacter baumannii.* [133] Thus, this high specificity enables targeted antibacterial activity but requires precise matching to patient isolates (**Supplementary figure 1**). Fortunately, due to the widespread presence of phages, a suitable natural phage for most clinically relevant bacteria can usually be rapidly identified in the environment. [7,111,118,152-157]




*Supplementary figure 1:* **Schematic illustration of bacterial host specificity: comparison of antibiotics and phages.** The host range of an antimicrobial agent refers to the spectrum of bacterial strains or species it can infect or kill. Most phages exhibit high host specificity, typically infecting only a limited number of strains within a single bacterial species (e.g., 20% of *E. coli* isolates tested). Some phages are termed "broad host range phages" because they can infect a larger proportion of strains within a single species (e.g., 50% of *E. coli* isolates tested). In contrast, broad host range antibiotics generally act across multiple bacterial species (e.g., various Gram-negative bacteria). Thus, the term "broad host range" has a different implication for phages than for antibiotics. Created with Biorender.com.

We recommend identifying the target bacteria using cultural methods, which allow for species identification and antimicrobial susceptibility testing. Specific phenotypes (e.g., small colony variants) or resistance mechanisms may be identified by standard clinical microbiology techniques. Genetic sequencing of the target bacterium is not a prerequisite for phage treatment but helpful for in-depth evaluation of treatment progress, if available.

*Background R2-5:* **The susceptibility of the target bacterium to phage(s) should be confirmed as close to treatment initiation as possible.**

In a case series of 785 requests for phage therapy, of which 17 patients ultimately received phage therapy, the median time between request and phage administration was 170.5 days (IQR 28-386). [158] Some bacterial species may develop adaptations under antibiotic selection pressure within days, which may affect their susceptibility to phages. Therefore, the susceptibility of the target bacterium to phage(s) should be confirmed as shortly before treatment initiation as possible. In time-critical situations, phage therapy can be initiated before phage susceptibility results are available. As soon as these are available, phage therapy should be adapted to the results.

*Background R2-6 – R2-8:*

- **To identify the target bacteria for phage treatment, all relevant clinical information should be correlated with available microbiological results on a case-by-case basis. Ideally, this discussion should involve infectious diseases specialists and microbiologists. In the case of a polymicrobial infection, we recommend identifying the lead pathogen(s) responsible for the infection.**
- **The time interval between sample collection and start of therapy should be as short as possible. If renewed pathogen detection is not successful, phage therapy should be based on the last pathogen identification result.**
- **Phage therapy may be considered in life-threatening situations, even if phage susceptibility is unknown. This approach may be considered if broad-spectrum phage cocktails are available.**

In some cases, the cultural isolation of the causative pathogen can fail. In these circumstances, attempts should be made to obtain confirmatory cultures prior to phage treatment, if this is clinically justifiable. If it remains impossible to identify the pathogen with a new sample analysis, personalized phage therapy is not feasible.

In addition, there is a possibility that over time, the patient's bacterium is no longer susceptible to the initially tested phage. This could occur due to mutations within the strain or the emergence of another strain. However, if clinical signs of infection persist and a repeated sample analysis prior to therapy initiation fails to re-identify the pathogen, treatment could be performed with the previously identified effective phage(s), if there is a persistent indication for phage therapy. The time interval between the identification of the pathogen, the phage susceptibility assessment, and the start of therapy should be as short as possible and corresponds to the basic principles of standard antibiotic therapy.

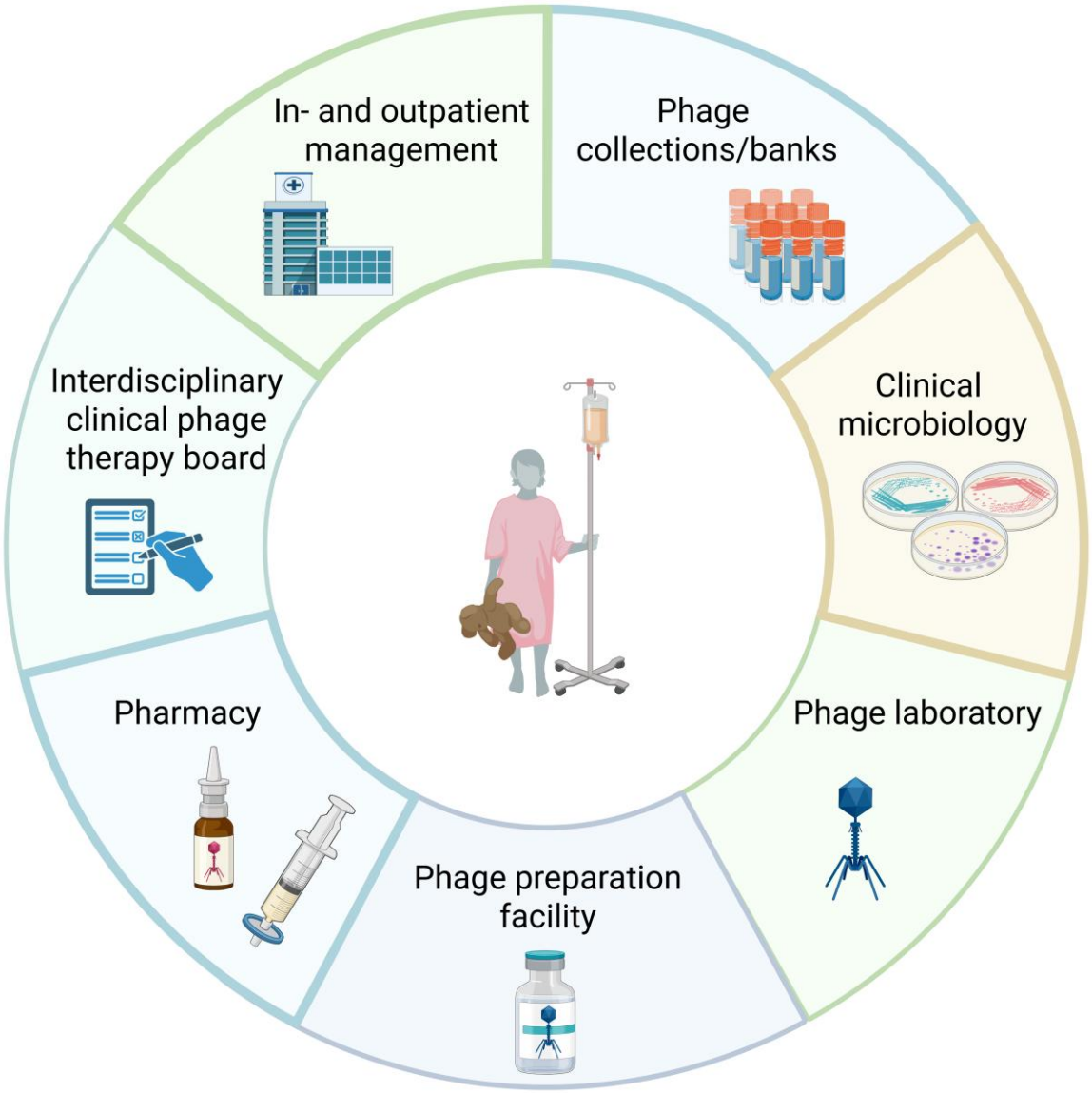
*Background R2-9:* **In case of various different bacterial species responsible for infection pathogenesis, all of them should be sent for phage susceptibility testing.**

Polymicrobial infections present a particular challenge for phage therapy, as phages exhibit narrow host ranges and are effective only against susceptible bacterial strains. When several species and/or distinct strains of the same species contribute to disease pathogenesis, untreated components may sustain infection despite targeted therapy against a single organism. Comprehensive phage susceptibility testing for all implicated bacteria is therefore critical. This ensures that therapeutic decisions are based on a complete understanding of which pathogens can be effectively targeted by available phages, thereby improving the likelihood of clinical success in infections involving multiple species and/or distinct strains within a single species.

# Supplementary explanation 1: Recommendations on infrastructure of personalized bacteriophage therapy

*Background S3-1:* **Personalized phage therapy requires an association with a dedicated infrastructure composed of the following interconnected modules:**

- **Phage collections/banks**
- **Clinical microbiology**
- **Phage laboratory**
- **Phage preparation facility**
- **Pharmacy**
- **Interdisciplinary clinical phage therapy board**
- **Clinic**




*Supplementary figure 2:* **Overview of the recommended infrastructure for personalized phage therapy, consisting of interconnected modules.** Created with Biorender.com.

Establishing a robust infrastructure for personalized phage therapy is essential to ensure its safe and efficient administration. This infrastructure involves several key components as mentioned in **S3-1** and **Supplementary figure 2**, that work together to provide comprehensive support for phage therapy.

The "phage laboratory" is not necessarily a dedicated phage research laboratory, but serves multiple purposes, including the testing of phages such as performing plaque and spot assays and liquid coincubation assays.

Common methods for phage counting and susceptibility testing include the plaque assay, spot assay, and liquid infection assays. The plaque assay, also called double agar overlay method and widely used to measure phage concentration (phage titer) in plaque-forming units (PFU)/mL, involves co-plating bacteria and phages, and observing the formation of clear zones, or "plaques," where phages have lysed bacterial cells. The spot assay is an approach in which phages are applied directly in serial dilution onto a bacterial lawn; clear or turbid spots indicate zones of bacterial lysis, offering rapid insights into

susceptibility. For the liquid infection assay, bacteria and phages are co-incubated in liquid culture and growth-curves following phage infection are observed and analyzed. Similar to an antibiogram, a phagogram based on the methods described above can be generated to assess bacterial susceptibility towards different phages. Another standard term related to phage susceptibility is host range determination, which involves testing phage infectivity across various bacterial strains. Standardization of phage susceptibility methods is of utmost importance to allow integration into microbiological diagnostics prior to patient administration. To date, the reproducibility of testing methods within laboratories and across laboratories is low. [134] The EUCAST (European Committee on Antimicrobial Susceptibility Testing) has recently established a subcommittee that is focusing on providing standardized approaches for this matter. [77, 159]

The term "phage preparation facility" refers to the dedicated space and equipment required for the manufacture of phages. It is important to note that such a facility may not necessarily need to comply with GMP standards for personalized phage therapy, although adherence to appropriate quality control and safety regulations is essential, as detailed below.

An "interdisciplinary clinical phage therapy board" is a group of healthcare professionals, including physicians (specialists relevant to the specific medical field alongside infectious disease experts), microbiologists, pharmacists, and possibly other experts, who collaborate to assess, plan, and manage phage therapy cases. This board provides a multidisciplinary approach to ensure that patients receive the most appropriate personalized treatment based on their clinical condition and microbiological profile.

While not all of these interconnected modules are strictly necessary for every administration of phage therapy, they form the ideal structure for a comprehensive, well-coordinated therapeutic approach. Importantly, not all components need to be housed in a single location, and some, such as the phage preparation facility, could be outsourced to specialized centers.

The performance of phage treatments should not be restricted to specialized medical centers. However, any center or institution performing phage treatments should meet minimum requirements for performing phage treatments and/or be connected to a supporting team with necessary infrastructure (i.e., a coordinating center) and adhere to recommendations for phage therapy as indicated in this guideline. It is likely that competence centers will emerge as phage therapy becomes available and should serve/be available as a reference to any center performing phage therapy. A definition for a competence center could be established (e.g., 10 treatments of a specific clinical indication) to support their development and identification as such.

The minimum requirements for medical centers performing phage treatments include appropriate storage facilities for phage preparations and medically qualified personnel to manage key aspects of treatment – such as phage administration, safety monitoring, administration of concomitant therapies, and microbiological sampling – aligned with the clinical indication and the chosen route of administration. While it is advantageous that centers have additional capacities to perform phage assays (e.g., susceptibility testing and phage-antibiotic synergy testing), these activities may be performed by external parties and associated results provided to the treatment center.

*Background R3-1 – R3-2:*

- **The minimum requirement for physicians starting phage treatment should be board certification.**

- **When phage therapy is started, at least one board-certified physician should be physically available for supervision, while the expertise required for additional input may be external.**

Medical education generally follows a harmonized structure and is divided into two main phases: basic medical training and specialist medical training, ultimately leading to board certification as a specialist physician. Basic medical training includes supervised theoretical and practical education. This phase provides future physicians with foundational scientific knowledge, clinical understanding, and supervised hospital experience. Specialist medical training, which follows the successful completion of basic training, involves full-time, hands-on participation in clinical activities within accredited hospitals or medical teaching institutions, under the supervision of national authorities. The minimum duration of this phase varies by specialty. Successful completion leads to board certification in a medical specialty.

*Background R3-3:* **Physicians who perform phage treatments should be encouraged to participate in either national or international networks.**

Should professional training opportunities regarding phage therapy become available, physicians should be encouraged to participate in such events. Training opportunities are offered, for example, by the European Study Group for Non-Traditional Antibacterial Therapy (ESGNTA) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). [160] Several global networks are already established to support and advance phage therapy and research, providing valuable platforms for collaboration, knowledge sharing, and clinical administrations, ensuring access to the latest developments and international insights. Among these (in alphabetical order):

- **Africa Phage Forum** [161]: An initiative that supports the administration and research of phage therapy in Africa, aiming to address public health challenges and enhance access to treatments.
- **PhageNET DZIF** [162]: An interdisciplinary German consortium that promotes translational phage research and its administration, working to integrate phage therapy into clinical practice and drive innovation in the field.
- ESGNTA [160]: The Phage Study Group within the ESCMID, which focuses on phage therapy research, providing a platform for collaboration among international experts in the field.
- Global Clinical Phage Rounds [163]: An international platform for presenting and discussing clinical cases of phage therapy, fostering cross-border exchange of knowledge and experience.
- Phage Directory [164]: A platform dedicated to facilitating the search for phages, helping researchers and clinicians identify appropriate phages for specific bacterial infections.
- Phage4-1Health [165]: A European consortium focused on dedicated phage experts and European research proposals related to phage therapy.
- Phagistry [78]: A forthcoming platform designed to document individual cases of phage therapy. Once launched, it will provide a structured system for recording and evaluating clinical experiences and outcomes, contributing to the growing body of knowledge on phage administrations.

*Background R3-4:* **Phage therapy should be documented, and data should be referred to a central registry if available.**

Phage therapy cases, including treatment regimens, outcomes, as well as unexpected/adverse events or organizational complications should be documented comprehensively in central phage registries, if available. The usual procedure in medicine for documenting unforeseen/adverse events should be

followed here, including the documentation of related clinical data. In the case of adverse events, the manufacturer of the phage preparation should be informed.

*Background R3-5:* **Physicians performing treatment with phages should document their cases in a patient registry.**

This documentation is essential for reporting and analyzing treatment protocols, outcomes, potential adverse events, and hurdles, thus helping to optimize phage administrations in the future. Registries such as Phagistry [78] (see Background **R3-3**) provide a structured approach to documenting patient outcomes, allowing for consistency in the information gathered. International registries enable the inclusion of cases from different regions. One challenge with international registries is the potential for information loss, especially due to inconsistent reporting resulting from varying standards or practices in data entry, or when data is entered in non-native language platforms. Additionally, ensuring that patient data is anonymized and protected from unauthorized access is critical, which can be challenging, especially when data is shared across borders with varying levels of privacy protection laws. Maintaining and updating a national or international registry requires significant resources, including funding, dedicated personnel, and infrastructure. Furthermore, physicians and researchers must allocate time to input data accurately, which can be burdensome, particularly in resource-limited settings. Despite these challenges, ensuring high-quality data collection and minimizing loss of information in personalized phage therapy cases will be essential for the global success of phage therapy.

*Background R3-6:* **Phage collections might make their phages accessible for scientific and potential therapeutic purposes.**

This guideline does not claim to stipulate the purpose of phage collections. However, we suggest that phage collections aim at providing phages for research purposes to a wider scientific circle. Phage collections can also decide to make their phages available for (potential) therapeutic purposes, irrespective of the size, composition, or classification of the collection. Phage collections may or may not be directly associated with downstream preparation processes.

*Background R3-7:* **We recommend that all phage collections be considered as phage providers for phage treatment.**

A phage API (active pharmaceutical ingredient) is a purified preparation of a single phage. Phage Therapy Medicinal Products (PTMPs) are formulations consisting of one or more such APIs, optionally combined with excipients, and intended for administration via various routes. [16]

The term "phage supplier" does not denote a clear distinction between someone who provides phages to test for susceptibility (e.g., the function of phage collections) and someone who prepares the phages as phage APIs. We tend to use "phage provider" for phage collections providing phages in any state of characterization but not at the stage of phage APIs.

Any collection should be considered a potential source of phages for treatment purposes. Given equal activity, better-characterized phages should be prioritized.

*Background R3-8:* **Phage collections should provide clonal phages and - as a minimum requirement - information on the host strain of this phage.**

This guideline is not intended to regulate phage collections and therefore can solely make propositions. Quality control measures (titer, genetic safety etc.) should be the responsibility of downstream stakeholders (e.g., during preparation). Phage collections should provide clonally derived phages, the identity of the host strain, storage conditions, and any additional available information on the phage(s)-of-interest (e.g., host range, genomic sequence). be followed by all laboratories.

*Background R3-9:* **To facilitate/promote the connection of national/local phage collections, the introduction of a central management entity may be considered.**

The information concerning phage collections is the choice and responsibility of the individual collections. A connection is desirable but would require a central management entity where phage collections can voluntarily list their collections. A central management entity may want to record information such as the size and specificity of the collection, the degree of characterization, provided services (i.e., if they send phages for testing or if they perform initial screening), associated fees or contract and contact information.

*Background R3-10:* **We suggest the implementation of a harmonized template Material Transfer Agreement (MTA) for phage providers providing phages for treatment purposes.**

A template Material-Transfer-Agreement (MTA) should be made available, stipulating the intended use for therapy and removing all responsibility/liability from phage provider(s), but the individual entities exchanging phages may use their own documents. Receiving entities should be able to provide proof of their biosafety level clearance. A template MTA for phages to be prepared for therapeutic purposes can be found in the attachments of this guideline (**Supplementary file 2**).

*Background R3-11:* **Phage screening and phage susceptibility testing for target bacteria may be performed locally or centrally.**

Phage screening and phage susceptibility testing can be performed locally or centrally, depending on the required and available infrastructure (e.g., biosafety level) and the qualification of personnel. The main emphasis lies in the methodology of the screening process. Results and interpretation criteria should be provided.

*Background R3-12 – R3-13:*

- **Phage susceptibility testing methods should be harmonized between all laboratories performing phage susceptibility testing.**
- **We recommend that proficiency testing be performed.**

The overarching goal for phage susceptibility testing should be the standardization of validated methods (e.g., plaque assay). We prioritize the performance of proficiency testing to facilitate interlaboratory harmonization rather than the implementation of a national reference laboratory. Once standardized methods for phage susceptibility testing are available, laboratories are encouraged to adhere to them.

*Background R3-14:* **Local diagnostic laboratories should provide the target patient's bacterial isolate, as well as identification and antibiotic susceptibility profiles.**

Local diagnostic laboratories should provide bacterial species isolation and identification, as well as antibiotic susceptibility profiles. Should phage susceptibility testing become more standardized such as by respective guidelines of EUCAST. [77,159] the role of local diagnostic laboratories could expand to incorporate phage susceptibility testing/companion diagnostics.

*Background R3-15:* **We recommend that local diagnostic laboratories providing phage susceptibility testing for phage therapy establish standard procedures. Those standard procedures should be guideline compliant.**

Local testing for phage susceptibility and potency offers several advantages, particularly in the context of personalized phage therapy. One significant benefit is the elimination of the need for shipping samples to distant laboratories, which can be time-consuming and costly. Additionally, local testing allows for better control over sample handling and quality, reducing the risk of contamination or degradation during transport. Although the testing procedures vary for different bacteria and phages, establishing standard procedures may enhance consistency and reliability, further enhancing effective and safe phage preparation. Guidelines may be issued by the EUCAST [77,159], and the general chapter 2.7.38, "Bacteriophage Potency Determination," is anticipated to be published in the near future.

## Supplementary explanation 3: Recommendations on preparation and quality control of personalized bacteriophage therapy

The Ph.Eur. Bacteriophage Working Party published the General Chapter "Phage Therapy Medicinal Products" (5.31) in 2024, which came officially into effect with European Pharmacopoeia edition 11.6 in January 2025. This general chapter provides guidance on basic requirements for the preparation and quality control of phage therapeutics. [16] It is currently not legally binding in the EU but serves as informative guidance, also for non-GMP-grade phage preparation. However, as a brief two-page overview, it may lack sufficient detail on the preparation process, highlighting the need for more comprehensive guidelines tailored to patient-specific PTMPs, particularly in cases of unmet medical need. The absence of such detailed guidance represents one of the main obstacles to magistral phage preparation, leading to hesitation or, in some cases, uncontrolled preparation without adequate quality standards. Thus, many countries around the world have initiated regulatory efforts to improve access to personalized phage therapy. Australia provides a clear example, as access to phage therapy occurs under the *Standardised Treatment and Monitoring Protocol*, regulated through the Therapeutic Goods Administration's Special Access Scheme. [166] In the United States, phage therapy may be accessed through a single-patient Investigational New Drug application submitted to the Food and Drug Administration under the Expanded Access Investigational New Drug programme. [118,167] Moreover, the EMA (European Medicines Agency) guideline on quality aspects of phage therapy medicinal products is currently under development. However, its scope might be limited to marketing authorization applications; magistral preparations or patient-specific phages not intended for marketing authorization seem to be outside the remit of this EMA guideline. [85] Consequently, the EMA guideline's scope does not overlap with the guideline presented here. Additionally, the EU is currently reforming the pharmaceutical legislation and aims to introduce "adapted frameworks" to allow flexible, risk-based adjustments to regulatory requirements for specific classes of medicinal products, including elements such as Recital 90, Article 28, and Annex VII of the draft Directive. These adaptations could address phage areas such as GMP requirements, platform authorization for multiple active substances, or patient-specific modifications of phages, along with tailored quality standards for personalized, emergency-use,

short-shelf-life medicines. While the outcome of consultations and the timeline for enacting this legislation remain unclear, there is hope that it will create transformative opportunities, especially for advancing phage therapy. However, this reform might also only be legally binding for phage medicinal products that require a marketing authorization. According to the current status of the reform, phage APIs prepared in pharmacies for personalized therapy (magistral preparation) will not be subject to EU regulation in the future (Article 1(5)(a) of the draft Directive). The receiving pharmacy must comply with country-specific regulatory requirements, which may include official inspections - such as demonstrating the ability to perform identity testing and assuming full responsibility for the quality of the phages used. To enable pharmacists to prepare phages magistrally on prescription in a pharmacy, rather than obtaining them from external sources, they require precise, detailed, and practical instructions.

## Supplementary explanation - table 1: Consented definitions for phage preparation

*Supplementary explanation - table 1:* Consented definitions for phage preparation

| Term | Definition | Strength of consensus |
|---|---|---|
| **Phage collection** | A phage collection is an assemblage of clonal phage stocks. In contrast to master phage seed lots, phages stored in phage collections are not necessarily fully characterized and/or suitable for therapeutic use (e.g., temperate phages). Phage collections may be one possible source in the search for phages for medicinal purposes. | *Strong consensus** |
| **Phage seed lot** | Phage seed lots contain clonally derived and well characterized phages and constitute the starting material for PTMP preparation. They should be described in detail and comply with requirements for identity, microbial purity, phage purity, potency, and absence of detrimental genetic factors as described in *Starting materials for phage preparation,* unless otherwise justified and authorized. The master phage seed lot (MPSL, also referred to as master phage bank) is used to prepare all working phage seed lots, although it may be directly used for (small scale) preparation. A working phage seed lot (WPSL), also known as a working phage bank, is derived clonally from the MPSL and meets the same quality and characterization standards as the MPSL. WPSLs provide a renewable and consistent supply of phages used in the (large scale) manufacturing process. | *Consensus** |
| **Collection of phage seed lots** | A collection of phage seed lots (MPSL collection) is an assemblage of clonally derived and well characterized phage seed lots. They must be | *Strong Consensus** |

| Term | Definition | Strength of consensus |
|---|---|---|
| | described in detail as specified in *Starting materials for phage preparation.* | |
| **Phage therapy API (active pharmaceutical ingredient)** | The phage therapy API is a purified phage harvest prepared using a suitable host, preparation process and quality control (as described in *General phage preparation principles* and *Quality and safety measures*) to be used for the preparation of a PTMP. | *Consensus** |
| **Phage therapy medicinal product (PTMP)** | PTMPs are preparations of naturally occurring or synthetic or engineered phages used to treat bacterial infections. They may consist of a single active pharmaceutical ingredient or a combination of multiple APIs along with excipients. These products can be delivered via different administration routes and are offered in a variety of dosage forms. PTMPs must meet the requirements for identity, microbiological quality, phage purity, and potency. Depending on the respective preparation, dosage form and administration route, additional tests may be required. | *Consensus** |
| **Bacterial master cell bank (MCB)** | The MCB contains a clonally derived and well characterized bacterial strain suitable for PTMP preparation. It must be described in detail as specified in *Starting materials for phage preparation*. The MCB is used to prepare all working cell banks (WCB), although it may be directly used for (small scale) preparation. | *Consensus** |
| **Bacterial working cell bank (WCB)** | The WCB is a clonal derivative of a qualified bacterial master cell bank and complies with the requirements as described there (*Starting materials for phage preparation*). Working cell banks provide a renewable and consistent supply of cells used in the preparation process. | *Consensus** |
| **Collection of master cell banks (MCB collection)** | A collection of master cell banks (MCB collection) is an assemblage of clonally derived and well characterized bacterial strains suitable for PTMP preparation. They must be described in detail as specified in *Starting materials for phage preparation.* | *Consensus** |
| **Bacterial host strain** | Bacterial strains that can be infected by a specific phage are considered as their hosts. [168] Not all bacterial hosts are suitable for phage manufacturing, for example, due to detrimental factors. However, if | *Consensus** |

| Term | Definition | Strength of consensus |
|---|---|---|
| | no established MCB is available in a timely manner that enables efficient preparation, the clinical bacterial isolate may be used for PTMP preparation for the individual patient to be treated. | |
| **Prophage** | A prophage is a phage genome integrated into a bacterial genome which can be induced (e.g., by stressors) to enter the lytic cycle in which the prophage replicates, and infectious phages are released from the bacteria. [169,170] They are ubiquitous in bacterial cells and can drive evolution by serving as vehicles for the transfer of fragments of chromosomal DNA and other mobile genetic elements. Prophages can be lost upon induction or be transmitted to the descendants of the host during bacterial replication.<br>Coinfection between productively infecting phages and prophages may lead to recombination and mosaic evolution among phages. Additionally, prophages of different phage types can be present within a single bacterium (polylysogeny). | *Consensus** |
| **Mobile genetic elements (MGEs)** | Prophage/ phage inducible chromosomal elements are examples of MGEs that use a variety of strategies to contribute to horizontal gene transfer, host adaptation, and virulence. Many MGEs provide an advantage for their bacterial host as they can encode virulence genes or toxins. Prophages can be induced by a number of stressors, including DNA damage and phage infection. Other subclasses are phage-inducible chromosomal islands (PICIs), phage satellites which hijack the virions of helper phages to disseminate. Satellite phages can also limit the propagation of their helper phage resulting in a certain level of phage immunity. | *Consensus** |
| **Shelf life** | The term shelf life defines the time period of a phage preparation stored in its original packaging under the recommended storage conditions, without chemical or physical changes that compromise its optimal performance. In manufacturing terms, shelf life refers to the time that elapses between the preparation date and the assigned expiration date. Some phage preparations that have passed their shelf life might still be safe and efficacious, but their quality is no longer guaranteed. | *Strong consensus* |

| Term | Definition | Strength of consensus |
|---|---|---|
| **Expiration date** | The expiration date is the final day that the manufacturer guarantees the efficacy and safety of the phage preparation. [171] The expiration date of a preparation is determined by stability studies and is a matter of regulatory approval for a license. | *Strong consensus* |
| **Phage concentration** | The phage concentration is defined as the phage abundance divided by the total volume of the API or PTMP. It refers to the total amount of phage particles without regard to their activity. Potential determination methods are qPCR and electron microscopy. | *Strong consensus* |
| **Phage titer** | The phage titer is defined as the functional phage concentration or dilution factor. It refers to the amount of phages that are capable of lysing their host bacteria. A potential method is the determination of PFU (plaque forming units)/mL on the propagation strain. | *Strong consensus* |
| **Microbiological quality of APIs/ PTMPs** | Sterile APIs or PTMPs are defined by the absence of viable micro-organisms confirmed by the test for sterility (Ph.Eur. 2.6.1) or using an alternative method with established equivalence (Ph.Eur. 2.6.27, 5.1.6). For non-sterile APIs or PTMPs, the microbiological quality is determined using a suitable method and complies with the established specification for the particular preparation. | *Strong consensus* |

*Consensus achieved during online survey

**The guideline proposes the following phage API/PTMP classification:**

1) Non-sterile API/PTMP

*a) Non-sterile API/PTMP* (Ph.Eur. 5.1.4)

- Microbiological quality: Microbiological thresholds according to Ph.Eur. 5.1.4 (Microbiological quality of non-sterile pharmaceutical preparations and substances)
- Administration: non-sterile administration routes, for example, oral, cutaneous (intact skin layer), nasal use

*b) Non-sterile API/PTMP with high microbiological quality* (Ph.Eur. 2.6.12; 2.6.27) [1]

- High microbiological quality: microbiological threshold of 0 CFU/mL or g
- Administration:
  - i.v. use in case of emergency (negative to date release)
  - administration routes other than non-sterile (e.g., wound administration)
  - short shelf life

2) Sterile API/PTMP

- Microbiological quality: sterility (Ph.Eur. 2.6.1, 5.1.6)
- Administration:
  - i.v. use and non-emergency cases
  - off-the-shelf preparations

The applicability and acceptance of the outlined approach of this guideline should be discussed in advance with the respective competent authorities (e.g., regional pharmacy inspectorate or health authority), and requirements may vary between countries.

**Supplementary explanation - table 2** provides the relevant details for quality and safety requirements for the magistral phage preparation.

*Background R4-1 – R4-6:*

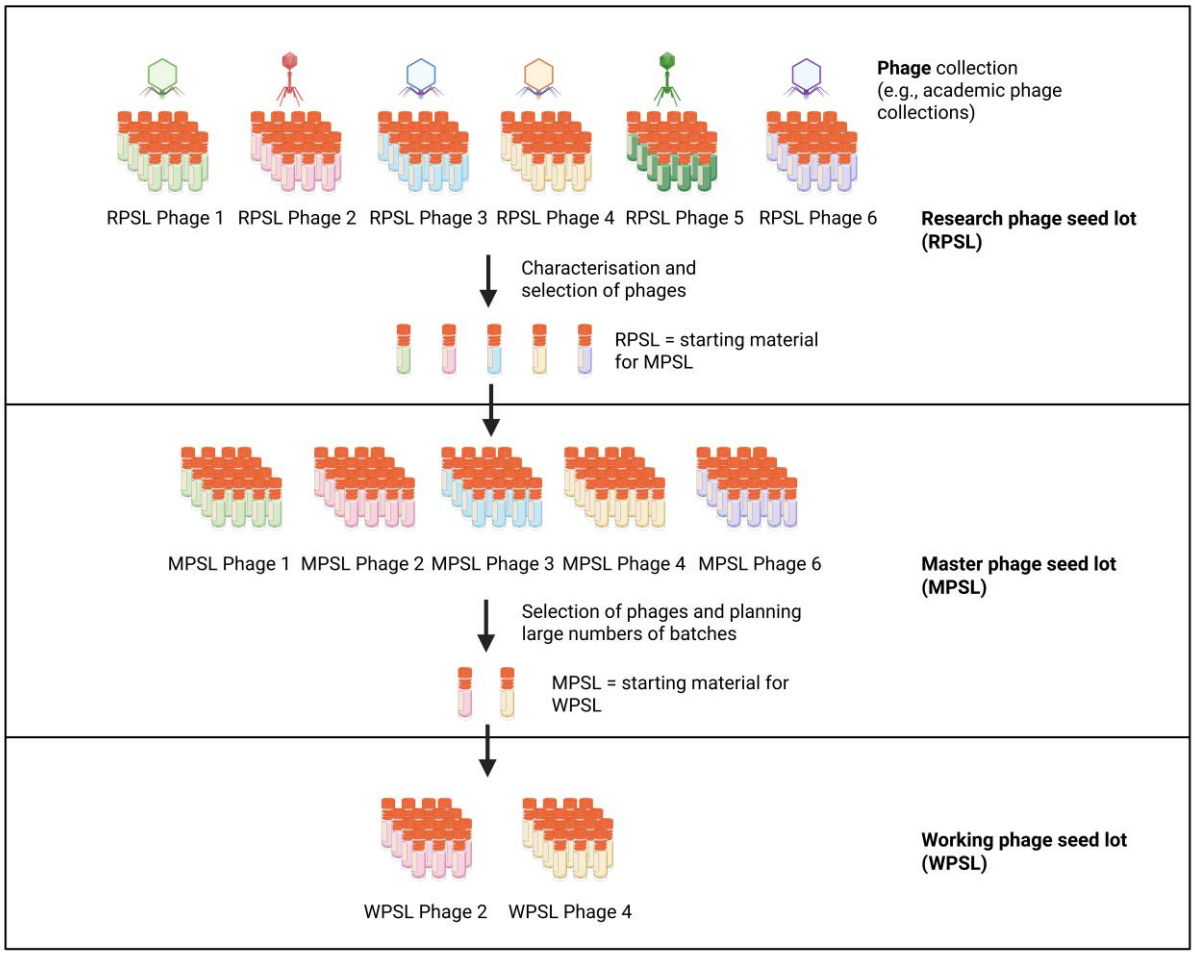
- **The search for suitable phages for the bacterial target can be performed using any suitable source, such as phage collections, collections of established phage seed lots, environmental sources, human sources including patient isolates, and synthetic and genetically engineered phages.**
- **Genetic modification of phage candidates, such as the removal of harmful determinants or lysogenic modules, can be used for phage therapy.**
- **During propagation in the context of phage preparation, preference should be given to bacterial host strains that do not contain potentially harmful genetic elements.**
- **Suitable bacterial propagation strains without prophages are rarely available but should be preferred for preparation whenever possible.**
- **If no other more suitable bacterial propagation strain is available, the respective patient isolate should be considered as a propagation strain for the individual patient.**
- **Phage seed lots used in PTMP preparation are clonal derivatives and should be characterized in detail (unless otherwise justified). This includes:**
  - **Information on the phage source, isolation location and year**

[1] This proposes a justified deviation from an EU recommendation, supported by the consensus process of this guideline. To ensure timely access to personalized phage therapy, it is essential to permit magistral preparation by qualified pharmacies. While these pharmacies may not meet all GMP requirements of industrial manufacturers, they have to follow defined and consensus-based quality standards such as provided by this guideline. This is in line with the procedure in pharmacies when preparing individualized medicinal preparations based on personalized prescriptions. We support this approach, provided it is accompanied by thorough documentation, clear justification, and transparent patient information.

- **Nucleotide sequence including the details of DNA/RNA extraction, sequencing, assembly, and annotation, with the respective year**
- **Results regarding antimicrobial/biocide/metal resistances, virulence, or lysogeny factors, with the respective year**
- **Known susceptible bacterial specie(s) and/or strain(s)**
- **Other parameters such as plaque morphology or phage morphology are determined, if relevant**

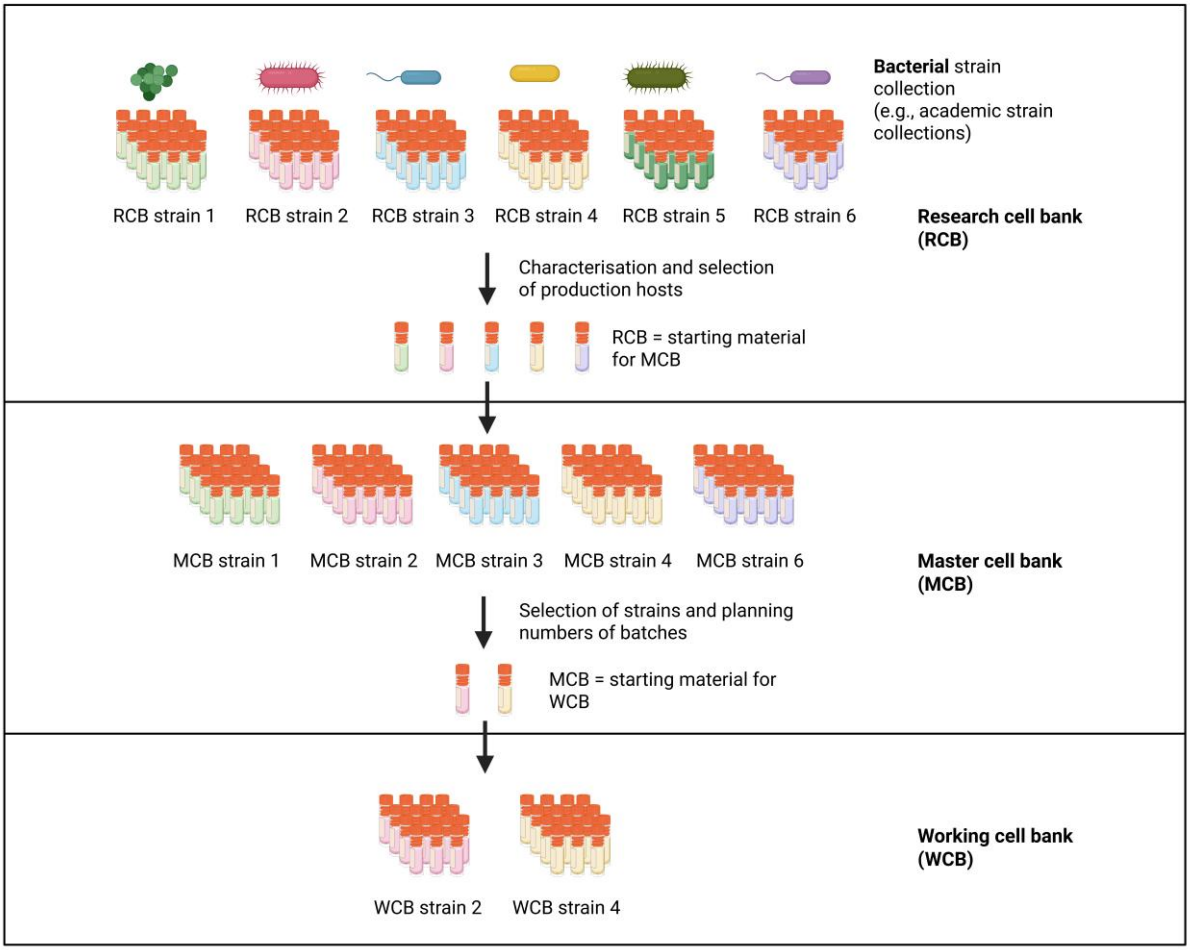
The preparation of APIs or PTMPs begins with well-characterized seed lot systems, employing a host-phage pair that has been demonstrated to be appropriate, unless a justified exception is provided.

**Phage starting materials** (**Supplementary figure 3**): Phage seed lots used in PTMP preparation are derived from a single phage clone and must be characterized in detail. Detailed information on the phage, including its origin, nucleotide sequence, and the bacterial species or strains it can infect, must be provided. Additional characteristics, such as plaque appearance or phage morphology, should be assessed when relevant. Phages that carry genetic elements associated with potential safety risks—such as antibiotic resistance genes or toxin-encoding sequences—should not be used unless a thorough risk assessment justifies their inclusion. In the case of genetically or chemically modified phages, the nature of the modifications and their impact on phage properties must be described. Master phage seed lots (MPSLs) employed in PTMP preparation must meet established requirements for identification, purity, microbiological quality, and potency. Any working phage seed lot used for preparation should be a clonal derivative of the MPSL and adhere to the same quality requirements as the MPSL. [16]




*Supplementary figure 3:* **Phage starting materials.** RPSL: Research phage seed lot. MPSL: Master phage seed lot. WPSL: Working phage seed lot. Created with Biorender.com.

**Bacterial starting materials (Supplementary figure 4):** All bacterial host cells used for the preparation of PTMPs must be fully characterized. This includes detailed information on the strain's origin, any subsequent manipulations, and the analytical methods applied for its assessment. Evaluation should cover the strain's antibiotic resistance profile as well as its complete genomic composition, including plasmid content. Strains carrying genetic elements that could pose safety risks—such as inducible prophages, toxin genes, or antibiotic resistance determinants—should be avoided. Bacterial cells intended for PTMP preparation must be derived from a clonal, well-characterized master cell bank (MCB) and comply with requirements for identity, phage susceptibility, microbial quality, viability, and absence of detrimental phages, unless a documented justification is provided for any deviation. [16] A working cell bank intended for preparation must originate as a clonal derivative of the MCB and meet the same requirements as those established for the MCB. [172] In case no established bacterial MCB devoid of detrimental factors is available for phage preparation, the clinical bacterial isolate may be used as a propagation strain for the treatment of the individual patient, if justified by a thorough risk assessment considering patient need and urgency.




*Supplementary figure 4:* **Bacterial starting materials.** RCB: Research cell bank. MCB: Master cell bank. WCB: Working cell bank. Created with Biorender.com.

*Background R4-7:* **Phage adaption should be an option to increase the efficacy of phage(s) to be considered for therapy.**

Phage adaptation (also referred to as phage adaption) is the process by which one phage or several phages can be directed to evolve on a clinical isolate from an individual patient in order to increase the antimicrobial spectrum and efficacy of the adapted phage preparation. [21,173]

From the adapted phage preparation, single phages can be isolated and seed lots can be prepared for further use. If the adapted preparation is directly used for therapy, it complies with the provisions for a non-adapted PTMP, unless otherwise justified.

Starting materials for phage adaptation are single phages or a mixture of phages that comply with the provisions for phage seed lots.

*Background S4-1:* **The standard method to obtain therapeutic phages is the culture-based method.**

**Overview (Figure 2 and Supplementary explanations figures 3-5):** In the classical culture-based method, therapeutic phages are obtained by propagating quality-controlled phage seed lots in defined propagation bacteria. This is followed by a purification process using appropriate methods preserving the biological properties of the phages and eventually resulting in a phage API. PTMPs are obtained by

the formulation of one or more phage APIs in a final dosage form. The choice of formulation is dependent on the indication and administration route. Excipients can enable efficient administration and enhance stability of the preparation. [16,80,85]

**Preparation of master cell banks (MCBs, Figure 2, Supplementary figure 4):** MCBs can originate from research cell banks (RCBs) or be directly derived from natural bacterial isolates from environmental or clinical samples and should enable efficient propagation of the phage of interest. Host bacterium isolates are the starting point for the preparation of MCBs by picking isolated colonies and cultivation in an appropriate volume of culture medium. The culture is mixed with cryoprotectant if required (in general glycerol) and stored in aliquots at a suitable temperature (e.g., -20°C for short term storage, -80°C for medium time span, in gas phase of liquid nitrogen for tong term storage). This MCB is used for the generation of phage seed lots and corresponding phage API preparation. The final MCBs comply with the requirements for identity, microbiological quality, viability, phage sensitivity, and absence of detrimental phages, unless otherwise justified. [16]

**Preparation of master phage seed lots (MPSLs, Figure 2 and Supplementary figure 3):** MPSLs originate from research phage seed lots (RPSLs) of natural phages isolated from environmental, clinical or other relevant sources or engineered phages. Optimal cultivation parameters (e.g., temperature, medium, duration, shaking frequency, MOI) for efficient phage preparation are specific to each phage-host combination. The MOI refers to the ratio of phages to bacterial cells in a given system. Preparation starts by serial dilution of the RPSL and plating in agar-overlay using a suitable concentration of top-agar and medium followed by incubation under appropriate conditions. A well isolated plaque from an agar-plate is transferred to a culture (e.g., 2 mL) of the host bacterium (corresponding MCB) using a sterile pipette tip. Based on this primary phage lysate, a suspension of appropriate volume (e.g., 100 mL) of the host (corresponding MCB) is infected with this primary phage lysate (generally MOI = 0.1 – 0.01) and incubated until cell lysis or sufficient phage propagation (secondary phage lysate). If the phage titer of the secondary lysate is not sufficiently high, a tertiary lysate should be obtained in analogy with the secondary lysate. The final phage lysate is filtered (0.2 µm), mixed with cryoprotectant (in general glycerol), and stored in aliquots at an appropriate temperature. The final MPSLs comply with the requirements identity, content, and purity. [16]

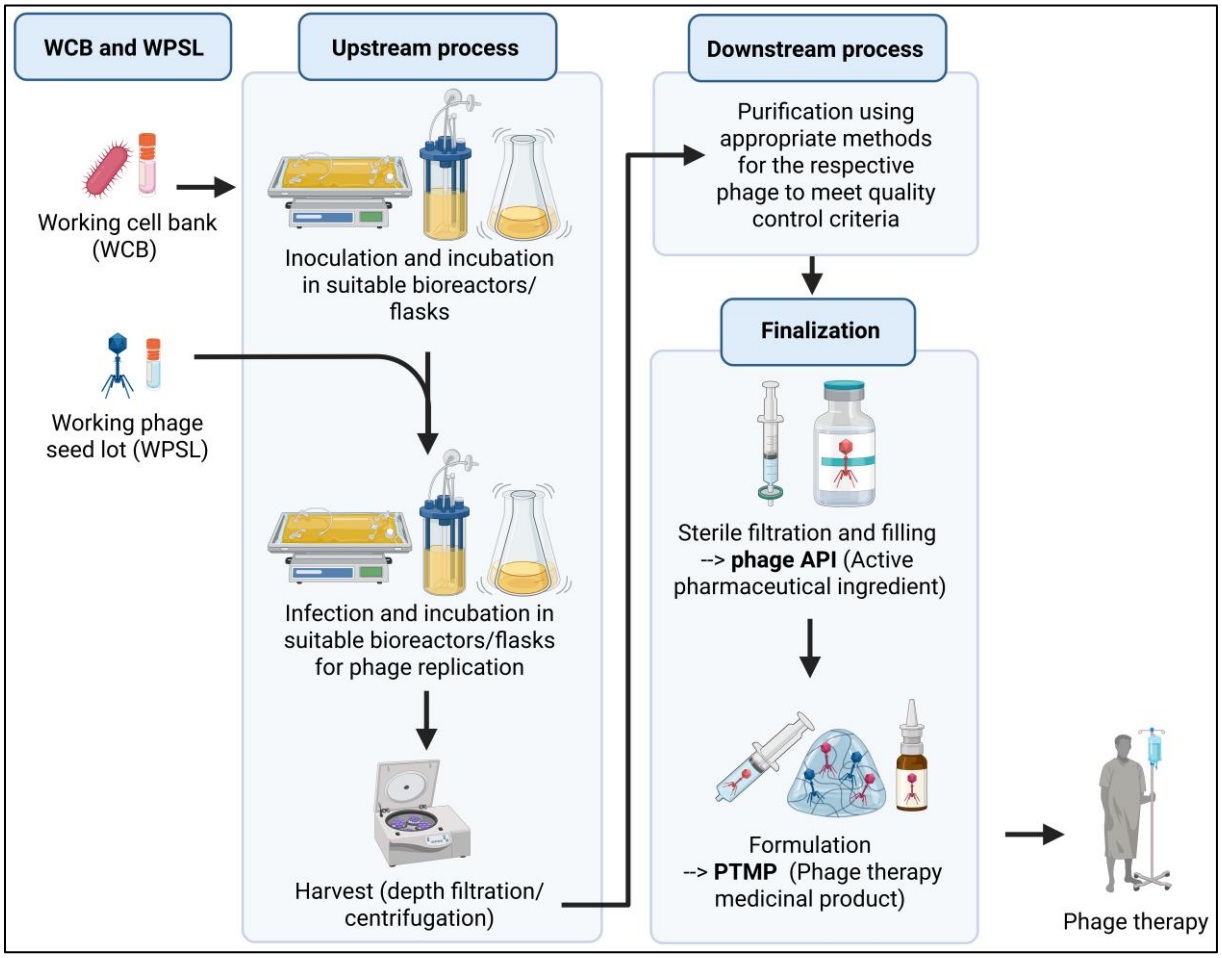
**Phage API**s are generated through propagation of MPSLs in MCBs, followed by a purification process employing suitable and validated methods that maintain phage biological activity while effectively reducing process- and host-related impurities, including host cell proteins and bacterial endotoxins. (**Supplementary explanations figure 5**). [16] Using a two-tiered seed lot system enables securing the stability of genetic and phenotypic properties. The preparation of phage APIs is divided into a cultivation upstream processing (USP) and a purification downstream processing (DSP) part. Cultivation parameters (e.g., temperature, medium, duration, agitation, aeration rate, MOI) allowing efficient phage propagation are specific for each phage-host combination. Cultivation takes place under defined conditions until the lysis of the host culture is completed or the phage titer is satisfactory. Different types of cultivation strategies can be used like batch or continuous bioreactor operations for larger volumes. Where suitable, small volumes of phage lysates can be obtained by cultivation in Erlenmeyer flasks or on semi-solid systems. Phage harvest includes the removal of cell debris and process related impurities by filtration or centrifugation steps, where applicable. Additionally, the level of impurities including

pyrogens is minimized using appropriate purification steps targeting host cell proteins and endotoxins. In general, chromatographic methods such as anion exchange chromatography or endotoxin affinity columns are used. Other methods such as ultracentrifugation, ultrafiltration, usage of organic solvents, or detergent treatment may also be used, but might require subsequent control and quantification of the used substances in the final preparation. The bioburden of purified phage APIs should finally be reduced by means of filtration through at least one 0.2 µm membrane. Membrane integrity must be verified after filtration. The final phage API complies with the requirements for identity, quantity/potency, microbiological quality, residual reagents, host cell impurities and contaminants (e.g., endotoxins), and (optionally) pH. Limits for these requirements should be determined upon a thorough risk assessment for different administrations and indications. [16,80-84]

**PTMPs** are obtained by the formulation of one or more phage APIs in a final dosage form for a dedicated administration or indication. Excipients can enable efficient administration and enhance stability of the preparation. The manufacturing process must be comprehensively documented in written procedures and protocols, including but not limited to equipment, materials, culture media, additives, culture conditions, and manufacturing steps. All procedures must be appropriately validated to demonstrate that the process consistently produces PTMPs meeting predefined quality specifications. [174]

The manufacture of phage APIs/PTMPs derived from MPSLs should be conducted in an environment that minimizes the risk of contamination. The applicable biosafety level (BSL) is determined by the host bacteria used in the production process (BSL-2 in most cases). Equipment and materials used for the preparation of phage APIs/PTMPs are designed, qualified, and maintained for their intended purpose, ensuring minimal risk to both personnel and recipients. All critical equipment and devices should be identified, qualified, and subject to regular inspection and preventive maintenance in accordance with the manufacturers' specifications. Where equipment or materials influence critical process or storage parameters (e.g., temperature, pressure, particle counts, or microbial contamination levels), such parameters should either be continuously monitored with defined alert and alarm limits and corresponding corrective actions, or the final preparation must undergo appropriate downstream testing (e.g., endotoxin testing) to verify that the critical parameters were maintained within acceptable limits. [17,23] Critical equipment is regularly maintained, serviced, cleaned, disinfected, and sanitized, with all activities appropriately documented. [23] All materials and reagents with direct contact with phage APIs/PTMPs are qualified for the intended purpose and are defined in form of specifications.

In particular, detailed specifications are established for culture media, additives (e.g., solutions and excipients), and primary packaging materials. Wherever feasible, culture media and additives free of animal-derived components should be used. When materials of human or animal origin are employed, appropriate measures should be implemented to control endogenous and adventitious agents, including transmissible spongiform encephalopathy (TSE) agents. Materials derived from TSE-relevant animal species are subject to a risk assessment demonstrating that all relevant TSE risk factors have been considered and that the risk has been minimized in accordance with the principles outlined in the Ph.Eur. general chapter 5.2.8 and the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01). [16,80,81,85,86]




*Supplementary figure 5:* **Example of a culture-based phage preparation**. WCB Working cell bank. WPSL Working phage seed lot. API Active pharmaceutical ingredient. PTMP Phage therapy medicinal product. Created with Biorender.com.

*Background S4-2:* **Synthetic systems provide an alternative to culture-based methods to prepare phage APIs and PTMPs.**

Phage assembly in a cell-free system is mimicked using transcription/translation systems (TXTL) derived from bacterial cell extracts combined with reagents/supplements (buffer containing nucleotides, amino acids, and reagents to support ATP recycling) to synthesize phage proteins encoded by a genomic DNA template. [145-147,152,175,176] This enables rapid phage preparation and the manufacture of designed phages, making the search for a suitable preparation host no longer necessary. In addition, engineered strains can be used to manufacture cell extracts that are endotoxin-free, which simplifies phage purification. Cell-extracts are multi-component and multi-parameter systems with considerable challenges with respect to standardization and robustness as well as controllability.

*Background R4-8:* **Quality control measures for APIs and PTMPs should include identity, potency, appearance, sterility/ microbiological quality, and purity (residual reagents, host-cell impurities, contaminants). If applicable, pH (liquid PTMPs), water content (solid PTMPs), and pyrogenicity should also be controlled.**

APIs and PTMPs require robust quality control measures to ensure their safety, consistency, and suitability for clinical use. Attributes such as identity, potency, appearance, sterility or overall microbiological quality, and purity are foundational elements of API and PTMP characterization and are universally recognized as essential indicators of product quality. Additional product-specific characteristics may be critical depending on the intended use of the material: pH can influence the stability and composition of liquid PTMPs, water content can affect the integrity of solid PTMPs, and pyrogenicity poses a particular risk for materials destined for parenteral applications. For PTMPs intended for intravenous application, assessment of hemolytic activity could be considered on a case-by-case basis as a precautionary approach, although the relevance and utility of such assessments are not yet well established and supporting evidence remains limited. Establishing robust control of these parameters provides a foundation for consistent manufacturing performance and ensures the safety and quality of the final medicinal product. Further details are available in **Supplementary explanations - table 2**.

*Background R4-9:* **To meet the respective clinical needs, quality requirements of the APIs and PTMPs can be adapted depending on the indication, administration, and formulation.**

Quality requirements for APIs and PTMPs must be sufficiently flexible to address the specific clinical context in which they are used. Although core attributes such as identity, potency, purity and microbiological quality form the basis of standard control, the stringency and breadth of testing may reasonably vary with the indication, route of administration and formulation. In particular, situations of clinical urgency, or circumstances in which phages must be propagated directly on patient-derived bacterial isolates, may necessitate deviations from conventional quality control paradigms. In such cases, risk-adapted approaches - while maintaining an appropriate level of assurance for safety and performance - can allow timely access to therapies that would otherwise be delayed by inflexible testing requirements. Any such deviation should, however, be formally justified and documented in writing, clearly specifying the rationale, the nature of the deviation, and the stage at which it is applied.

*Background S4-3:* **Qualified laboratories using validated methods are needed to perform quality control measures on APIs and PTMPs.**

Qualified laboratories – not referring to an official certification or regulatory qualification but phage and laboratory expertise as well as adherence to this guideline - can perform quality control of phage APIs and PTMPs. Testing is performed on retained samples that are not intended for patient administration. Mostly, a BSL-2 laboratory without cleanroom conditions is sufficient for this purpose, provided that appropriate procedures and expertise are in place.

*Background R4-10:* **Various formulations and administration routes exist for phage preparation and the selection should be based on the specific indication and patient characteristics.**

Formulation options are diverse [87,88] and include:

- Liquid formulations: e.g., infusions, suspensions for nebulization [89,90]
- Solid formulations: e.g., (coated) tablets, encapsulations, lyophilizates, nanofibers [91-97]
- Semi-solid formulations: e.g., ointments, creams, hydrogels, aerosols [36,98-102]
- Phage coating: e.g., stents, grafts, catheters [103,104]

Possible routes of administration described in thousands of published cases or clinical trials include, amongst others:

- Topical (e.g., intraoperative, cutaneous)
- Intravenous (i.v.), including peripherally inserted central catheter (PICC) line use
- Intra-articular
- Inhalational
- Intravesical
- Rectal
- Vaginal
- Intrathecal
- Intraperitoneal
- Oral
- Nasal
- Ophthalmic

The different routes of administration might also be used in a combination. [21,41,177-179]

*Background R4-11 – R4-12:*

**The preparation label should state [105]:**

| API | PTMP |
|---|---|
| **Name of the phage** | **Name of the phage(s);**<br>**possibly other excipients** |
| **Titer (in PFU/mL) and total content (in mL)** | **Titer (in PFU/mL); possibly strength of other excipients; total content** |
| **Date of preparation**<br>**Expiration date (or: recommended shelf life)**<br>**Typical target bacteria** | **Date of preparation**<br>**Route of administration**<br>**Expiration date (or: recommended shelf life)**<br>**Typical target bacteria** |

**The following information should be provided in the accompanying documentation (supplementary sheet) [83,110]:**

- **Qualitative and quantitative composition, including**
  - **Phage identity**
  - **Phage provider/manufacturer and contact details**
  - **Phage quantity (titer in PFU/mL) including date of (last) titering**
  - **Identity of the host bacterium/bacteria used in the preparation process (to the strain level)**
  - **Typical target bacteria**

  - **If available: additional information on the host range**
- **Pharmaceutical form**
- **Recommended storage conditions** [2]
- **Preparation date**
- **Expiration date (including after opening),**
- **Instructions for reporting serious adverse reactions and/or events**
- **Instructions on how to dispose of unused (expired) phage preparations**
- **For dried preparations:**
  - **the name, composition, and volume of the reconstituting liquid to be added**
  - **the period of time within which the preparation is to be used after reconstitution**
- **Presence of residues known to be harmful (e.g., antibiotics, ethylene oxide)**
- **Presence of phage and/or host characteristics known to be harmful (e.g., damaging genetic determinants, lysogeny, transduction, prophages)**
- **Bacterial residues such as (endo)toxins**
- **Certificates of analysis of all components of the preparation**
- **Instructions for opening the container/package**
- **Publications referring to the phage**

APIs and PTMPs should be accompanied by both a preparation label and a supplementary information sheet. The preparation label is a global label covering both outer and inner packaging and must allow for the unequivocal identification of the content [180]. Additional and more detailed information regarding the preparation should be provided in a supplementary sheet, supplied separately from the packaging.

*Background R4-13:* **The stability of the phage(s) in the respective preparation and administration mode should be ascertained.**

Another complexity of phage therapy is the biological nature of phages with a limited shelf life. Their concentration can decrease over a storage period of a couple of weeks to months, but also due to pharmaceutical formulations or administration methods, such as nebulization. [89,91,181-183]

The stability of the respective phage API and PTMP (here referred to as administration mode) should be tested using titering methods as described above. The following parameters may influence phage stability:

- Temperature
- pH
- UV light
- Ionic strength
- Purity
- Humidity in lyophilized APIs/PTMPs
- Adsorption to matrices and shear stress
- Phage concentration

[2] Ideally, the phage supplier should provide validated storage conditions for each phage (formulation). If data is lacking, general storage conditions should be indicated (e.g., storage in glass vials, temperature 4°C) with the notice that optimized storage conditions should be evaluated by the operating institution itself.

*Background R4-14:* **The appropriate storage conditions for APIs and PTMPs should be evaluated for each phage (formulation).**

The ideal storage conditions for each phage and formulation should be tested. If there is no data provided by the supplier, storage conditions should be evaluated by the operating institution itself using titering methods as described above. [83] Generally, glass vials (pharmaceutical glass type 1 (Ph.Eur.) should be used for storage to limit titer loss due to phage adsorption.

*Background R4-15:* **If a phage product is supposed to be stored for a longer time, the shelf life (or for unlicensed phage preparations: recommended storage time) should be determined for each phage and formulation according to individual stability tests, including phage activity, microbiological quality, pH, and, where relevant, water content and excipients.**

*Shelf life* refers to the time period during which the API or PTMP maintains its specified phage titer, microbiological quality, pH, moisture level, and, when applicable, excipient content within established limits. [184] In manufacturing terms, shelf life refers to the time that elapses between the preparation date and the assigned expiration date.

The *expiration date* marks the last day on which the manufacturer assures that the phage preparation maintains its full potency and safety. [185] The expiration date of a preparation is determined by stability studies and is matter of regulatory approval for a license.

For unlicensed APIs or PTMPs used in personalized phage therapy, in absence of a (licensed) expiration date, the *recommended storage time* should be indicated by the manufacturer.

*Background R4-16 – R4-17:*

- **Unlicensed APIs or PTMPs used in personalized phage therapy may have their recommended storage time extended if their potency and microbiological quality are guaranteed by thorough retesting.**
- **If unlicensed APIs/PTMPs are stored beyond their recommended storage time, their microbiological quality and efficacy should be verified by periodic determination of the following parameters:**
  - **Phage titer**
  - **Microbiological quality**
  - **pH**
  - **Where relevant, stability of supplementary preparations such as excipients**

Some unlicensed API or PTMPs that have passed their recommended storage time might still be safe and efficacious, but their quality is no longer guaranteed. If further use is considered after the recommended storage time of APIs or PTMPs, the potency and microbiological quality of the phage preparation should be ascertained by re-testing using titering methods as described above.

*Background R4-18:* **Supplementary explanation - table 2** is **R4-18**.

The manufacturing process for GMP-grade phage APIs is time-consuming, with a waiting period of several months to obtain the final product, and the cost can exceed €100,000 for every new phage manufacturing. In certain countries, manufacturing costs might even be higher, as experienced during the preparation of the IMP for the clinical trial Phage4Cure in Germany with estimated manufacturing costs of 2 million Euro for three GMP-grade phages. [111] Due to the high specificity of phages, with sometimes only one matching phage per patient that may not be beneficial for other patients, the potential biological evolution in patients' disease-causing bacteria during the manufacturing process, the frequent need for therapy adjustments during phage treatment, and the time-critical aspects of some patient cases, GMP-manufacturing of phages for personalized phage therapy is not feasible for most patient requests. Drawing on our experience, only a small subset of patients is likely to benefit from GMP-grade phages for individualized treatments. This might mainly be the case when suitably matched GMP-grade phage APIs authorized for use outside clinical trials are available, such as those produced within the FORMPHAGE project in Frankfurt, Germany. [186]

Magistral phage preparations, while exempt from industrial GMP requirements under Directive 2001/83/EC, must still meet quality parameters such as microbiological quality in line with the state of scientific and technical knowledge. This guidance outlines the scientific and technical knowledge for magistral preparation of phage APIs and PTMPs on a named-patient basis, drawing on relevant EU regulatory frameworks. Germany is used as a national example.

Explanation of room requirements for phage preparation:

- Phage propagation and purification, including initial sterile filtration, is mostly performed in a biosafety level 2 (BSL-2) laboratory or equivalently controlled environment. While this does not require a cleanroom, it must be operated by personnel with high expertise in phage work.
- Sterile filtration and filling: In the absence of a more suitable regulatory framework, our guidance on air quality has been mainly based on the European Guide to Good Manufacturing Practice. However, since magistral phage preparation is not subject to GMP compliance, deviations may be acceptable, provided that alternative environmental controls ensure an adequate level of air cleanliness. Such setups must be justified (e.g., through appropriate particle monitoring) and agreed upon in consultation with the authorities responsible.
- Quality control of phage APIs and PTMPs, which is the testing of retained samples that are not administered to the patient, can be performed in, e.g., a BSL-2 laboratory, provided appropriate phage expertise is in place.

## Supplementary explanation - table 2: Magistral phage preparation: quality and safety requirements

*Supplementary explanation - table 2:* Magistral phage preparation: quality and safety requirements. This table is R4-18 (A, strong consensus)

| **Characteristics** | **Requirements** | **Test procedures / methods** | **Specifications/ comments** | **Grade of recommendation** | **References** |
|---|---|---|---|---|---|
| **Preparation environment** | | | | | |
| **Air quality** (in terms of particle and microbial colony counts) | Air quality requirements for sterile filtration and aseptic filling | Air quality requirements for sterile API/PTMP<br>- Equivalent to Grade A<br>- Background environment<br>equivalent to a minimum of grade B for Restricted Access Barriers Systems (RABS); respectively grade C or D for isolators | Sterile filtration and aseptic filling must be performed in an environment where the air quality, in terms of particle and microbial counts, meets Grade A standards as defined in the current European GMP Guide (Annex 1) and Directive 2003/94/EC. The surrounding background environment should meet Grade B requirements for RABS and Grade C or D for isolators. | Strong recommendation for sterile phage APIs/PTMPs<br>Open recommendation for non-sterile phage APIs/PTMPs [a] | - European Guide to Good Manufacturing Practice, Annex 1 [23]<br>- General monograph version I phage active pharmaceutical ingredients<br>- Directive 2003/94/EC<br>- RiliBÄK corneal transplant, ch.5 [187] |

[a] In non-sterile APIs/PTMPs (Ph.Eur. 5.1.4) and non-sterile APIs/PTMPs with high microbiological quality, air quality requirements may be reduced after risk assessment. Expanding the potential reduction of air quality specifications for the conceived category of non-sterile APIs/PTMPs with high microbiological quality proposes a justified deviation from EU recommendations and is supported by the consensus process of this guideline.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Safety requirements** (Exposure of workers to biological agents) | Biosafety containment level | - Dependent on the bacterial host strain used in the preparation process<br>- Phages: S1 (provided they do not carry known undesirable genes and are not genetically modified) | - | Strong recommendation | - European Directive 2000/54/EC<br>- European Directive 2009/41/EC (genetically modified phages) |
| **Precautions** | Avoidance of release of phages and bacteria in the preparation facility and environment | Inactivation of biological waste taking into account the properties of different phage types (e.g., autoclaving, UV radiation, disinfection) [188] | - | Strong recommendation | [188] |
| **Equipment, materials, and preparation process** | | | | | |
| **Maintenance** | Validation, regular inspection | In accordance with the manufacturer's instructions | - | Strong recommendation | - |
| **Monitoring** | Depending on the equipment, e.g., temperature, pressure, particle counts, and microbial contamination levels | Alarms, calibration, regular service, cleaning, and disinfection | - | Strong recommendation | - |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Preparation process** | Detailed description | - Detailed information on materials, equipment, culture media, supplements, cultivation conditions, purification processes, packaging components, and other relevant items.<br>- Validation methods<br>- Documentation<br>- SOPs | - | Strong recommendation | - Directive 93/42/EEC of 14 June 1993<br>- Directive 98/79/EC of the European Parliament<br>- Council of 27 October 1998 |
| **Culture media** | Animal component-free culture media and additives. If animal product-free media are not used, TSE-free certification should be obtained. | - | - | Strong recommendation | EMEA/410/01 |
| **Phage seed lot: see also 4.1. Specific definitions for phage preparation** | | | | | |
| **Origin** | Known origin:<br>Document pedigree/history (e.g., isolation source and host) | Review of scientific literature, laboratory notebooks, consignment letters, MTAs, and other relevant documents. | - | Strong recommendation | - |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | If the phage is sourced from a supplier: include the accompanying documentation provided by the supplier. | | | |
| **Identification** | Identification at the species level [b] | Full genome sequencing and analysis [c]<br>- Mapping reads to the reference phage sequence should yield an identity over 99%.<br>- Analysis for prophage contamination or other phage-inducible elements. | Residual fragmented bacterial DNA can be expected but should be investigated for prophage excision. | Strong recommendation | 189-195 |
| **Lytic growth** | Confirmation of lytic growth / Avoidance of lysogeny modules if possible. [d] | Full genome sequencing: Genome analysis for the presence of integration/excision genes and for genes associated | Advantages of lytic phages:<br>- more potent killers of host bacteria than temperate phages | Strong recommendation<br>Possible exemption: | 189,200<br>Ph.Eur. 5.31 |

[b] Characterization steps may be carried out before the preparation process, e.g., by the phage provider.
[c] Next generation sequencing (NGS) or Third generation sequencing (such as Nanopore sequencing) for identification are the preferred methods. They additionally allow for the obligatory screening for known undesired genes and bacterial DNA.
[d] Lytic phages utilize the bacterial host's cellular machinery to replicate and ultimately cause cell lysis, releasing newly formed phage particles. In contrast, temperate phages integrate their genetic material into the host bacterium's chromosome, forming a lysogen in which the phage genome exists as a prophage and replicates along with the host without killing it. Under specific conditions, temperate phages can be induced to enter the lytic cycle, producing new phage progeny.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | with a temperate life cycle [e] (e.g., genes encoding integrases, regulatory elements controlling integrase expression, and sequences with integrase-like functions). [f] | Limitations of temperate phages:<br>- Temperate phages might transfer fragments of host bacterial DNA (transduction, horizontal gene transfer).[196,197]<br>- Temperate phages might carry antibiotic resistance genes or toxin genes that act as virulence factors for lysogenized bacteria. [197,198]<br>- Bacteria that have been lysogenized may develop resistance to lytic infection by the phage that established the lysogeny. [199] | Use of lytic derivatives of temperate phages (bioengineered phages that lost genomic integration ability) [200] can be considered. | |
| **Potential of conferring toxicity, virulence or** | Confirmation of absence of known or potentially harmful genetic determinants (e.g., toxicity, virulence, or | Full genome sequencing: [h] Genome analysis for the presence of known toxin genes or genes transferring virulence | The quality of the genome analysis is highly dependent on | Strong recommendation | Ph.Eur. 5.31 |

[e] Sequence annotation cannot provide guarantees since not all functions of all the gene products are yet known.
[f] If lytic growth has already been demonstrated on the reference phage (e.g., MPSL collection), a sequence comparison with subsequent analysis of non-matching reads may be sufficient.
[h] If the absence of known or potentially damaging genetic determinants has already been demonstrated on the reference phage (e.g., MPSL collection), a sequence comparison with subsequent analysis of non-matching reads may be sufficient.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **antibiotic resistance** | antibiotic resistance) [g], if possible. | factors or antibiotic resistance with indication of the database used. [189] | the sequencing method and the database(s) used. | | |
| **Phage quantification** | Quantification of phages<br><br>For the storage of MPSL, higher phage titers (e.g., > 1e9 PFU/mL) are advantageous (stability). | *Phage titer* (biological activity):<br><br>Functional phage concentration, dependent on the host bacterium.<br><br>- Double agar overlay method (DAO) [169,201]<br><br>*Phage concentration:*<br><br>Phage abundance divided by the total volume.<br><br>- Methods to determine the phage concentration can only lead to an approximation of the infectious titer and should not be used exclusively: qPCR/RT- | For phage preparation, the phage titer is the relevant parameter, not the phage concentration.<br><br>Recommended procedure for phage titering: DAO or spot test in increasing dilutions on the propagation strain in biological triplicates. | Strong recommendation | [169,202]<br><br>General monograph version I phage active pharmaceutical ingredients<br><br>Ph.Eur. 5.31; 2.7.38 "Bacteriophage potency determination" |

[g] Detrimental genetic determinants are potentially harmful even in supposedly strictly lytic phages as not all gene sequences are fully described yet and one thus cannot be sure of the strictly lytic nature of phages. Additionally, there is the possibility that bacteria produce toxin/virulence factors before they have been lysed by their phages.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | qPCR; [202,203] droplet digital PCR (ddPCR); [204] flow cytometry; [205] mass spectrometry; [206] transmission electron microscopy [207] | | | |
| **Phage API (Phage active pharmaceutical ingredient): see also main document Table 3** | | | | | |
| **Purification** | Purification procedures need to be designed to minimize the content of harmful bacterial components, pyrogens, and culture medium residues. | Multiple purification options exist. [84,208]<br><br>A validated method should be used, e.g., sterile filtration, affinity columns, tangential flow filtration, gel filtration, cesium chloride banding, ultrafiltration, or ultracentrifugation. If necessary, further steps after purification have to be performed to eliminate chemicals used for purification. | Endotoxin removal is advisable for both Gram-negative and Gram-positive bacteria when endotoxin levels exceed acceptable thresholds. [i]<br><br>The use of harmful chemicals such as chloroform on medicinal phage preparations should be avoided. The permissible daily exposure of chloroform is 0.6 mg according to ICH harmonized guideline Q3C(R6). If used, measurement of chloroform is mandatory.<br><br>If cesium chloride is used, a dialysis step will have to be | Strong recommendation for Gram-negative bacteria<br><br>Regarding endotoxin removal in Gram-positive bacteria: open recommendation | [83,84,169,208]<br><br>General monograph version I phage active pharmaceutical ingredients<br><br>ICH harmonized guideline Q3C(R6)<br><br>Ph.Eur. 5.1.13 |

[i] The rationale for Gram-positive bacteria is the risk of contaminated tips or media. However, the use of clean media, water, and consumables is crucial.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | | performed to eliminate it from the final phage preparation. [84,208] | | |
| **Identity/ genetic stability** | Identification (confirmation that the correct phage was prepared and the preparation is not contaminated with another phage). | Full genome sequencing [j] followed by a comparison to the sequence of the starting material. No new analysis is necessary if the sequence conforms to that of the starting material. In case of phage training, the resulting phage is considered a new seed lot. | Regarding safety, the quality of mutated sequences may be more significant than their quantity. For instance, single nucleotide polymorphisms or indels in phage genes typically pose no safety risk, unlike the acquisition of harmful host genes through recombination or transduction. [k] | Strong recommendation | [189,191,209,210] |
| **Potency** | The recommended phage titer of an API depends i.a. on the planned formulation, administration route, indication, and patient characteristics (e.g., weight). It is essential to determine and document the titer of the phage API. | Standard: Determination of the infectious phage titer with the DAO method | - | Strong recommendation | [169,202]<br>General monograph version I phage active pharmaceutical ingredients<br>Ph.Eur. 5.31; 2.7.38 "Bacteriophage |

[j] Full genome sequencing is preferred over other genotyping techniques because it is more effective in detecting potential contamination.
[k] Transduction occurs when phages encapsulate bacterial DNA—either chromosomal or plasmid—and transfer it to a different bacterium.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | | | | potency determination” |
| **Microbiological quality** | Sterile APIs or PTMPs are defined by the absence of viable microorganisms confirmed by the test for sterility (Ph.Eur. 2.6.1) or using an alternative method with established equivalence (Ph.Eur. 5.1.6). In the case of non-sterile APIs or PTMPs, the microbiological quality is evaluated through suitable testing procedures and is required to conform to the established specifications for that preparation.<br><br>For PTMPs, sterility (Ph.Eur. 5.1.1; 2.6.1) might be required for some administrations depending | *A) Methods for sterility testing* (absence of viable microorganisms)<br><br>- Test for sterility: Membrane filtration method based on the European Pharmacopoeia (Ph.Eur. 2.6.1). Problem: time-consuming, laborious, expensive, potentially titer-reducing for big phages<br><br>- Alternative microbiological methods with established equivalence to Ph.Eur. methods (Ph.Eur. 5.1.6) | Not every new phage preparation necessarily requires individual microbiological quality testing, provided the process of sterile filtration and aseptic filling remains unchanged and has been previously validated. In-house microbiological quality testing is permitted if personnel are appropriately trained in aseptic microbiological techniques, the testing is conducted under aseptic conditions (e.g., laminar airflow in a controlled environment), and the methods comply with standards such as the European Pharmacopoeia 2.6.1, and are fully documented. In practice, some EU member states, such as Germany, allow in-house microbiological quality | Open recommendation:<br><br>Sterile APIs/PTMPs in the following situations<br><br>- i.v. use and non-emergency cases<br><br>- Off-the-shelf preparations | - EU GMP guidelines specific to Advanced Therapy Medicinal Products<br><br>- General monograph version I phage active pharmaceutical ingredients<br><br>- Ph.Eur. 2.6.1; 5.1.6; 2.6.12; 5.31; 2.6.27 |

| **Characteristics** | **Requirements** | **Test procedures / methods** | **Specifications/ comments** | **Grade of recommendation** | **References** |
|---|---|---|---|---|---|
| | on the route of administration, e.g., intravenous.<br><br>However, for some situations (e.g., limited time in emergencies) or administration routes (e.g., local), sterility testing according to Ph.Eur. 5.1.1/ 2.6.1 may not be feasible, and the microbiological quality could be specified by other suitable methods (Ph.Eur. 2.6.12). In this case, although the preparation cannot be claimed to be sterile, it can conform with the required microbiological quality. | | testing if the pharmacy can demonstrate the validity and adequacy of its procedures. Alternatively, microbiological quality testing can be outsourced to a qualified external microbiological laboratory.<br><br>The guideline proposes the following phage API/PTMP classification:<br><br>*1) Non-sterile API/PTMP*<br><br>*1.a) Non-sterile API/PTMP according to Ph.Eur. 5.1.4*<br><br>- Microbiological thresholds according to Ph.Eur. 5.1.4 (Microbiological quality of non-sterile pharmaceutical preparations and substances)<br><br>- e.g., for oral, cutaneous (intact skin layer), or nasal use | | |
| | | *B) Methods for determination of microbiological quality*<br><br>- e.g., Ph.Eur. 2.6.12, 2.6.13, 2.6.27 | | Strong recommendation:<br><br>Non-sterile APIs/PTMPs need to comply with the specifications as in Ph.Eur. 5.1.4.<br><br>Open recommendation:<br><br>Microbiological quality with a threshold of 0 CFU/mL or /g for non-sterile API/PTMP with high microbiological quality | Ph.Eur. 2.6.12, 2.6.12; 2.6.13; 2.6.27; 5.1.4 |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | | *1.b) Non-sterile API/PTMP with high microbiological quality* [l]<br>- Microbiological threshold of 0 CFU/mL or g<br>- i.v. use in case of urgency [m]; administration routes other than non-sterile (e.g., wound administration); short shelf life<br>*2) Sterile API/PTMP*<br>- Sterility according to Ph.Eur. 2.6.1/5.1.6<br>- i.v. use and non-emergency cases; off-the-shelf preparations | | |

[l] This proposes a justified deviation from an EU recommendation, supported by the consensus process of this guideline. To ensure timely access to personalized phage therapy, it is essential to permit magistral preparation by qualified pharmacies. While these pharmacies may not meet all requirements of industrial manufacturers, they have to follow defined and consensus-based quality standards such as provided by this guideline. We support this approach, provided it is accompanied by thorough documentation, clear justification, and transparent patient information.

[m] In special situations (e.g., emergency), it may not be possible to wait for the final sterility result (Ph.Eur. 2.6.1.) of the finished preparation before its release. In these cases, after risk assessment, alternative methods for preliminary results may be used while the final preparation sterility test incubates for the full 14-day duration, even after the preparation has been administered to the patient. If the results of the sterility test of the preparation are not available at release, mitigation measures should be implemented, including informing the treating physician. Prior to application, it must be ensured that a sterilization step (filter size 0.22 µm, if compatible with phage biology) with no subsequent exposure to the environment has been performed.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Pyrogenicity** | Quantification of endotoxins/ pyrogens [211]<br><br>As an API is not directly applied to a patient (it will be further processed to a PTMP), no fixed API endotoxin threshold is required. Nevertheless, the endotoxin level needs to be quantified and indicated on the API to be able to calculate the final endotoxin level in the PTMP. The PTMP needs to conform with the required threshold compatible with the administration as defined by Ph.Eur. 5.1.10. | - Recombinant Factor C test (rFC) (Ph.Eur. 2.6.32). [212] The rFC test mostly replaced the Limulus Amebocyte Lysate (LAL) assay, which had raised ethical and environmental concerns.<br><br>- Monocyte activation test (Ph.Eur. 2.6.30). Advantage: additional detection of non-endotoxin pyrogens as it measures the IL-6 production. [213,214] | In accordance with the above suggested phage API/PTMP classification:<br>*1) Non-sterile API/PTMP*<br>*a) Non-sterile API/PTMP according to Ph.Eur. 5.1.4*<br>- No pyrogen threshold [n], e.g., for oral, cutaneous (intact skin barrier), or nasal use<br>*b) Non-sterile API/PTMP with high microbiological quality*<br>- Pyrogen threshold < 5 EU (endotoxin units)/kg of body weight per h [o]. However, in this category exemptions may be possible based on risk assessment.<br>- i.v. use in case of emergency; administration routes other than | Strong recommendation | [18,83,212,213]<br><br>Ph.Eur. 2.6.14; 2.6.30; 2.6.32; 5.1.10; 2.6.27; 5.1.13 |

[n] Although there is no fixed threshold for pyrogen levels in non-sterile phage APIs/PTMPs, a purification step should be performed as the risk of adverse reactions (e.g., rash) may be elevated with unpurified phage preparations.
[o] Parameters needed for the calculation of endotoxin thresholds include the endotoxin level of the API, the dilution factor in the PTMP, dosage and patient weight.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | | non-sterile (e.g., wound administration)<br>*2) Sterile API/PTMP*<br>- Pyrogen threshold < 5 EU/kg of body weight per hour (Ph.Eur. 5.1.13)<br>- i.v. use and non-emergency cases; off-the-shelf preparations | | |
| **Genomic purity** | Detection of genetic impurities: e.g., host cell DNA, microbiological contamination by other bacteria, fungi, or extrinsic phages | Full genome sequencing (recommended method with advantage of detection of unexpected/unknown DNA-sequences): mapping reads to the propagation strain chromosome and to the predicted prophage(s). | No fixed threshold of percentage of reads matching the desired phage. It depends on the quality (not quantity) of the genomic impurity. Decision based on individual risk assessment. | Strong recommendation | Ph.Eur. 2.6.35 |
| **Protein contamination** | Contamination level of detrimental proteins, e.g., toxic or hemolytic proteins | Detection of bacterial proteins using for example:<br>- ELISA<br>- SDS-PAGE + Western Blot | Host cell proteins are absent or within the established specification.<br>If the MAT assay is used for pyrogenicity testing, | Open recommendation | Ph.Eur. 5.31 |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | - Antibody kits for specific proteins where available (e.g., for hemolysins) | determination of protein contamination can be omitted. | | |
| **Prophages/ temperate phages** | Determination of prophages/temperate phages.<br><br>Accepted prophage level depends on propagation host: [p]<br>*1. Standard propagation host*<br>*1.a)* Standard propagation host; prophages devoid of detrimental sequences: While no definitive threshold has been established due to limited data availability, a high relative abundance may | Full genome sequencing of the phage API: The genetic analysis of a phage API should include the detection of sequences that map to the predicted prophage region(s) within the propagation bacterium. On suspicion of prophage replication and copurification with the target phage, visual inspection for a coverage peak in a prophage coding region and assessing the relative abundance is recommended. To estimate the relative abundance, one can compare, for example, the median coverage of phage API reads mapping to one predicted prophage region of the | Random fragmentation of the bacterial DNA can be expected and should not pose any safety concern. However, in case the propagation host is not the patient isolate, if prophage contamination is observed, an additional analysis of the prophage genome(s) needs to be carried out to ensure absence of known toxin/AMR genes.<br>If no known harmful genes are present, the contamination by prophage(s) could be considered an impurity that will probably not negatively impact the activity of the therapeutic lytic phage - as it was able to replicate in | Strong recommendation<br><br>Optional for propagation on patient bacterial isolate | - |

[p] Phage preparation on the bacterial strain usually used by the preparations facility for a specific phage (standard propagation host) in contrast to phage preparation on a clinical isolate of the patient to be treated.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | indicate a potential prophage impurity.<br><br>*1.b)* Standard propagation host; prophages contain detrimental sequences: Exclusion of the batch for phage therapy. Exceptions may be possible after risk assessment (e.g., emergency).<br><br>2) Patient bacterial isolate Propagation on patient clinical isolate and phage administration only to the patient, even if prophages contain harmful sequences: No prophage threshold since the propagation strain and all its prophages are already present in the patient. Any contamination with the same prophages is not expected to present an additional risk for the patient. The | propagation host to the median coverage of phage API reads mapping to the target phage sequence. | presence of this prophage - nor pose any danger to the patient. | | |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | determination of prophages is not obligatory in this case. | | | | |
| **pH** | pH measurement | pH test (EP method) | Desired pH level depends on the administration, typically pH 6 – 8. [83,108,215] | Strong recommendation | Ph.Eur. 2.2.3 |
| **Appearance** | - Absence of visible particles in the phage solution (Ph.Eur. 2.9.20)<br>- Opalescence (Ph.Eur. 2.2.1)<br>- Coloration (Ph.Eur. 2.2.2) | EP method, CPMP-ICH guideline | The API complies with the established specification. | Strong recommendation | - Ph. Eur 2.2.1; 2.2.2; 2.9.20; 5.31, Supplement 11.6<br>- CPMP-ICH guideline |
| **Hemolytic activity** | Test for hemolytic activity | F756-13 ASTM Standard Practice for Assessment of Hemolytic Properties of Materials | - | Open recommendation if phages are intended for i.v. use<br>Optional in case of emergency | F756-13 ASTM Standard Practice for Assessment of Hemolytic Properties of Materials |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Residual reagents** | The API should be evaluated for the presence of residual preparation reagents that could have safety implications. | Depending on methods/chemicals used in the purification process<br>e.g., residual nuclease, cesium chloride, or organic solvents | - | Strong recommendation | Ph. Eur 5.31, Supplement 11.6 |
| **Phage therapy medicinal product (PTMP): see also main document Table 3** | | | | | |
| **Dilution, carriers, packaging** | - Dilution solutions, carriers, and packaging materials must meet documented requirements and specifications and when applicable the requirements of EU 2017/745 on medical devices<br>- Carriers must be chosen that allow the required phage activity during the intended administration period (stability). | - | - | Strong recommendation | EU 2017/745 on medical devices |
| **pH** | Desired pH level depends on the administration, typically pH 6 – 8. [83,108,215] | pH test (EP method) | - | Strong recommendation | Ph.Eur. 2.2.3 |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Appearance** | The API complies with the established specification. | EP method, CPMP-ICH guideline | - Absence of visible particles in the phage solution (Ph.Eur. 2.9.20)<br>- Opalescence (Ph.Eur. 2.2.1)<br>- Coloration (Ph.Eur. 2.2.2) | Strong recommendation | - Ph. Eur 2.2.1; 2.2.2; 2.9.20; 5.31,<br>Supplement 11.6<br>- CPMP-ICH guideline |
| **Potency** | The recommended phage titer of a PTMP depends i.a. on the planned formulation, administration route, indication, and patient characteristics (e.g., weight).<br>It is essential to determine and document the titer. | Obtained via formulation/dilution of a phage API | The titer of a PTMP is usually between 1e6 to 1e9 PFU/mL. *Comment: During the endorsement process, this recommendation required additional clarification, with a strong reference to the background text or R5-5.* | Strong recommendation | [169,202]<br>- General monograph version I phage active pharmaceutical ingredients<br>- Ph.Eur. 5.31; 2.7.38 "Bacteriophage potency determination" |
| **Phage mixtures (cocktails)** | - Phage combinations require careful attention to formulation parameters, as each phage within the | Potential measures include, for example: | Testing of phage synergy or antagonism is performed preclinically before the preparation process. Phage APIs | Open recommendation | - |

| **Characteristics** | **Requirements** | **Test procedures / methods** | **Specifications/ comments** | **Grade of recommendation** | **References** |
|---|---|---|---|---|---|
| | mixture may need a distinct formulation to preserve its potency and stability. Accordingly, assessments may be conducted to evaluate potential synergistic interactions or mutual inhibition, including risks such as phage aggregation, interference due to competition for the same bacterial receptor, or the development of cross-resistance. | Planktonic killing assay, [216,217] kinetic growth curves | can subsequently be combined to a PTMP if no antagonistic effects have been demonstrated. | In case of emergency, phages may be combined on an educated assumption and the according tests can be performed while the treatment is ongoing. | |
| **Combination therapy** | Testing of synergistic versus antagonistic effects in combination therapies (e.g., with antibiotics) | Potential measures include, for example:<br>Planktonic killing assay, [216,217] kinetic growth curves, checkerboard assay,<br>antibiotic disk + phage diffusion | Interaction testing is performed preclinically before the preparation process. | Open recommendation<br>In case of emergency, phages may be combined on an educated assumption and the according tests can be performed while the treatment is ongoing. | [60,151,218] |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Stability in combined preparations** | Testing of phage stability in combined PTMPs | Testing of titer with the DAO method after incubation with combination preparations after storage period | Importance of stability testing:<br>- Documented inactivation of phages in preparations with antiseptic/antibiotic activity and/or high acidity [219]<br>- As protein-based entities, phages are sensitive to conditions that can denature proteins, including exposure to organic solvents, elevated temperatures, extreme or non-optimal pH, variations in ionic strength, and interfacial stress. [108,215,220] Additionally mechanical stress (e.g., during formulation, encapsulation mixing, or spraying) can damage phages. [221,222] | Strong recommendation for stored PTMPs | [219] |
| **Bacterial working cell bank: see also main document Table 3** | | | | | |

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| **Origin** | Known origin:<br>Document pedigree/ history/ pathogenicity level of isolate | Review of scientific literature, laboratory notebooks, consignment letters, MTAs, Nagoya Protocol requirements, etc. Alternatively, if bacteria are sourced from a supplier: documentation accompanying the bacteria from the provider | - | Strong recommendation | - |
| **Identification** | Identification at the species (and strain) level<br>Possible exception of strain identification using NGS: patient isolate used for phage preparation only for the particular patient. | Full genome sequencing and analysis (prophage, AMR, toxins). Read mapping to the bacterial chromosome and to the prophage regions.<br>Other methods include for example mass-spectrometry, short read sequencing or 16S-PCR for species identification. | - | Strong recommendation<br>Optional for propagation on patient bacterial isolate | - |
| **Microbial purity** | Absence of microbial contaminants | Plating or any other suitable method (e.g., Ph.Eur. 2.6.36 | - | Strong recommendation | Ph.Eur. 5.31 |

| **Characteristics** | **Requirements** | **Test procedures / methods** | **Specifications/ comments** | **Grade of recommendation** | **References** |
|---|---|---|---|---|---|
| **Viability** | Determination of the number of viable cells | Plating and Colony Forming Unit counts of the phage preparation in respective conditions (e.g., LB at 37°C overnight) or any other suitable method (Ph.Eur. 2.6.36) | - | Open recommendation | Ph.Eur. 5.31 |
| **Phage sensitivity** | The susceptibility of the bacterial strain to the phage is verified | *Recommended procedures:*<br>- DAO method [q] with increasing dilutions of phage filtrate [223,224]<br>- Spot test [r] in increasing dilutions [225] | - Direct spot testing and culture lysis can have false positives (e.g., bacterial lysis caused by lysis from without) but are simple and good for automating. Thus, direct spot test or culture lysis can be used for screening. Positive tests should then be followed by titration to confirm permissive hosts. | Strong recommendation | [225,227-231]<br>Ph.Eur. 5.31 |

[q] The Double Agar Overlay (DAO) method involves mixing serial dilutions of phage filtrate with host bacteria and applying the mixture to a plate either by spreading or using a soft agar overlay. After incubation, plaques are assessed for their presence. This technique demonstrates productive phage replication, and the characteristics of the plaques can provide information on whether the phage follows a lytic or temperate life cycle. However, the method requires the host to grow to confluence on solid media, and not all phages produce visible plaques even in productive hosts, due to limited diffusion through the agar or low replication efficiency. If plaques are difficult to observe, agar can be replaced with agarose, which facilitates diffusion of larger phages in the softened medium.

[r] In the spot test, a bacterial lawn is established on a plate, and small volumes of phage filtrate are applied to its surface; following incubation, zones of lysis indicate the presence of phages. The “Direct spot test” (only 1 dilution) needs to be differentiated from the “spot test in increasing dilutions” (= semiquantitative). Only the semiquantitative approach should be used if no confirmatory plaque assay is planned. Advantages: Simple; allows testing of multiple phage filtrates on the same plate. Limitations: host must grow to confluence on solid media; prone to false positives due to bacterial killing by media components or phage binding that does not lead to a productive infection.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | | *Alternatives (not recommended as exclusive method):*<br>- Liquid culture/ planktonic killing assay [s] (culture lysis) [226]<br>- Linking phages [t] to their hosts by using genomic sequences [227-230] | - Host range testing by using genomic sequences is not yet sufficient to replace *in vitro* testing. For the moment, it is only acceptable as supportive information and/or screening aside from conventional susceptibility testing. | | |
| **Prophages/ mobile genetic elements** | Determination and indication of prophages/ mobile genetic elements [194,201,232-234] | Full genome sequencing and screening for potentially inducible prophage sequences or phage-like elements: [u] | Suitable bacterial propagation strains without prophages are rarely available. | Strong recommendation | [235,236,238]<br>Ph.Eur. 5.31 |

[s] Liquid culture (culture lysis): A phage filtrate is introduced into a bacterial broth culture and incubated, with lysis assessed by reduced turbidity or metabolic dye readouts. Advantages: Suitable for hosts that cannot form confluent lawns on solid media; readily scalable and compatible with automated, high-throughput phage screening. Limitations: May yield false positives from non-productive lysis; some phages infect efficiently on solid media but fail to do so in liquid culture.

[t] Associating phages with their bacterial hosts using genomic approaches—such as predicting host range from genomic similarity through k-mer comparisons (e.g., HostPhinder), oligonucleotide frequency pattern analyses, or receptor-binding protein sequence analysis—is not yet sufficiently accurate to replace traditional susceptibility testing. Nevertheless, these methods can be valuable for preliminary screening and for providing complementary supporting evidence.

[u] Prophages: Clinical wild type strains commonly have prophages. Many prophages modify the cell surface structures of their hosts (e.g., serotype conversion, lipopolysaccharide glycosylation and acetylation, wall teichoic acid glycosylation). Lytic phages have evolved to infect these wild type strains. Thus, some phages may not be able to be prepared in prophage free propagation bacteria, consequently reducing the number and diversity of available efficient phages.
In some cases, it may be necessary to use phages isolated directly from the patient's bacterial strain that cannot be propagated in standard prophage-free propagation hosts. However, because such personalized phage preparations are administered only to the same patient, any residual host bacterial DNA would be negligible compared with the amount already present in that individual. Thus, the determination and indication of prophages/mobile genetic elements may be omitted in those cases.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | No fixed threshold level for prophages/mobile genetic elements<br>If prophages are present, documentation and sequencing of prophages is obligatory. | (e.g., PhiSpy, PHASTEST) [235-237] | | Optional for the propagation on the patient's bacterial isolate | |
| **Toxin or antibiotic resistance genes** | Determination and indication of toxin or antibiotic resistance genes<br>During propagation in the context of phage preparation, strains containing potentially harmful sequences (e.g., antibiotic resistance determinants, toxins, virulence factors) should be avoided, if possible. [v] | Full genome sequencing | If no established bacteria can be found in a MCB in a timely manner, the clinical bacterial isolate (that might contain potential detrimental factors) may be used for API and PTMP preparation for that individual patient. | Strong recommendation<br>Optional for the propagation on the patient's bacterial isolate. | [18] |
| **Use of patient bacteria** | If no other suitable bacterial propagation strain is available, the respective | Species identification (see also above) and antibiotic susceptibility profile | If the strain used for preparation is the patient isolate, all potentially detrimental factors | Strong recommendation | Ph.Eur. 5.31, Supplement 11.6 |

[v] Can be omitted in case the patient isolate is used for phage propagation and the application is intended for this individual.

| Characteristics | Requirements | Test procedures / methods | Specifications/ comments | Grade of recommendation | References |
|---|---|---|---|---|---|
| | patient isolate should be considered as a propagation strain for the individual patient.<br>If the patient isolate is used for phage propagation for that individual patient, the characterization of this host may not require the same level of detail as for standard propagation host. | | are already present in the patient. Thus, characterizing does not offer increased safety to the patient. The only exception would be for toxins that might be copurified and concentrated with the phage during preparation and could be assessed with a specific ELISA kit (if available) or the MAT. [w] | | |

[w] This proposes a justified deviation from an EU recommendation, supported by the consensus process of this guideline. To ensure timely access to personalized phage therapy, it is essential to permit magistral preparation by qualified pharmacies. While these pharmacies may not meet all requirements of industrial manufacturers, they have to follow defined and consensus-based quality standards such as provided by this guideline. We support this approach, provided it is accompanied by thorough documentation, clear justification, and transparent patient information.

# Supplementary explanations 4: Recommendations on administration of personalized phage therapy

*Background R5-1:*

**The following aspects should be taken into account to properly plan phage therapy:**

- **Patient history, such as prior antibiotic therapy, previous interventions, and surgeries**
- **Previous therapy with phages**
- **Extent, localization, and severity of the infection that should be targeted using phage therapy**
- **Presence of prosthetic material, other implants or other foreign material in the body**
- **Underlying conditions, allergies, and co-medication including ongoing antibiotic and other anti-infective therapy**
- **Overall clinical status of the patient**

When considering previous phage therapy assessing the presence of anti-phage neutralizing antibodies may be a factor in selecting the most suitable phage(s). However, there is a knowledge gap regarding whether these antibodies may affect phage efficacy. [18,127,142,143,239]

*Statement S5-1 and Background R5-2:* **The choice of inpatient/outpatient setting should be based on an individual evaluation, including severity of the infection and general condition of the patient.**

**Supplementary file 3** presents a model document of an informed consent form to be adapted to local requirements and the patient's individual situation.

The decision whether the administration and monitoring of phage therapy should take place in an inpatient or outpatient setting should be based on a case-by-case assessment. This concerns in particular the start of phage therapy. Given that phages are still an experimental form of treatment, it is generally preferable to initiate treatment in an inpatient setting. Monitoring intervals might be extended, once the first dosages have been applied without complications. Following uneventful initiation of phage therapy in the inpatient setting, continuation at home — for example, within a framework of outpatient parenteral antimicrobial therapy or hospital-in-home programs — may be considered in suitable patients.

*Background R5-3:* **Close monitoring of vital signs, laboratory and microbiological parameters should be performed before, during, and after phage therapy as clinically necessary for the specific case.**

Bacterial resistance to phages can develop over time due to co-evolutionary processes, as bacteria adapt to the selective pressure exerted by phages (**Supplementary`explanations figure 6)**. [118,121-123,240-243] Thus, phage therapy often requires continuous monitoring and adjustment during treatment to remain effective in previous reports. [21,112,119,124,151,241,244]

To evaluate clinical effectiveness and safety, several monitoring parameters should be applied:

- Vital signs (such as breathing rate, heart rate, blood pressure, body temperature).
- Laboratory parameters that may be helpful in assessing severity of infection and response to treatment. This may include markers of inflammation and of organ failure.
- Microbiological assessments including culture (such as from sputum or tissue samples), to allow for repeated susceptibility assessments of the target bacterium and the phage selected for

treatment. When phage-resistant variants are found, which might have evolved under phage selection pressure, further characterization of these variants - including antibiotic susceptibility testing - can provide valuable insights for adapting the patient's anti-infective therapy.

- To assess whether phages are reaching the target site, PCR and phage titration of relevant samples - both with and without prior phage propagation - can provide valuable insights for optimizing the route of administration. Notably, qPCR values alone cannot represent phages activity.
- Changes in the immune response to phage exposure, including the presence of phage-neutralizing antibodies, could be analyzed through serum-phage co-incubation followed by titration assays. These findings may inform further phage selection and adaptation; however, as noted above, there remains a knowledge gap regarding the extent to which these antibodies impact phage efficacy.
- Vital signs should be monitored according to standard clinical routine, adapted to the individual patient's clinical status. For laboratory parameters, immune responses, and phage pharmacokinetics, one possible approach would be to collect samples on the day before treatment initiation, the day after, and on days 4 and 7, followed by further testing as clinically indicated. Additionally, confirmation of phage susceptibility using bacteria sampled during treatment could be performed at least one week after the start of therapy, and then, for example, on a weekly basis thereafter.

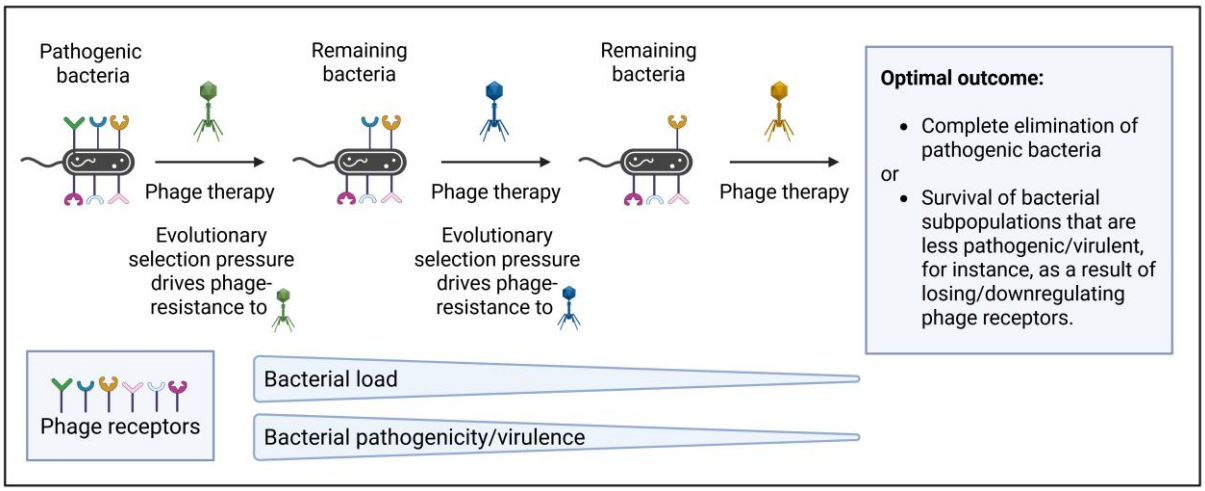
*Background R5-4:* **Given the current state of scientific understanding, standardizing administration methods is not feasible, resulting in the absence of a singular method for any given indication. Therefore, decisions regarding administration should be tailored to each patient's individual treatment plan.**

A personalized approach - tailored to the individual patient and discussed with experts - is recommended for phage therapy. Key factors include the phage composition and phage concentration of the dosages, the route of administration, the pharmaceutical formulation, the timing and frequency of administration, the duration of therapy, and the need for dynamic adjustments to these parameters throughout therapy. More evidence from RCTs is needed to determine standardized treatment plans for future phage administration.

The decision on phage composition in the dosage involves selecting which phages matched to the patient's bacterial strain(s) will be administered and whether they will be used as a single phage, in a sequential regimen, or as a phage cocktail. Phage cocktails include a composition of phages, for example, a 1:1:1 ratio for a three-phage cocktail. When using a phage cocktail, it is recommended to assess the stability of the phage combination as well as their potential synergistic effects or competitive interactions together with the patient strain, if feasible. [18,112-117] To maximize potential synergistic effects, selecting phages that target different phage receptors on the patient's bacterial strain may be beneficial. [18,112-116,245] If feasible, this selection should be carried out on the patient's specific strain, as phage receptors can vary even among bacteria of the same species from different patients.

While phage cocktails are a promising strategy, they may not always be the most effective approach for all clinical settings. Sequential phage therapy as displayed in **Supplementary explanations figure 6**, potentially (1) leveraging phage-driven evolutionary trade-offs and the subsequent weakened state of resistant bacteria, and (2) reducing phage resistance to the available phages at the same time, might sometimes outperform cocktail approaches. [118-120]

- Explanation for (1): Evolved phage resistance mechanisms can help bacteria evade phage therapy; however, they can come with evolutionary trade-offs that impair essential bacterial functions. The subsequent decrease in the bacterial strain's overall fitness and pathogenicity potentially improves clinical outcomes by making the bacteria less harmful or easier to treat such that either the human immune system in combination with antibiotics succeeds in eliminating them or that the surviving bacteria are driven to a commensal relationship with patients. [119-126]
- Explanation for (2): In a phage cocktail, there is a risk that only the most well-matched phage will initially be effective against the bacterial strain, while driving cross-resistance for accompanying phages as well as neutralizing antibodies. [18,127] This could ultimately result in a scenario where, once resistance also develops against the initially effective phage, no remaining suitable phage is available for treatment.




*Supplementary figure 6:* **Sequential phage therapy – a simplified overview.** The evolutionary pressure exerted by phages on bacteria can lead to the development of resistance to a specific phage, similar to how antibiotics drive the emergence of antibiotic-resistant bacteria. When bacteria become resistant to a given phage, a new, well-matched phage can be identified. This approach addresses the challenge of evolving bacterial resistance to ensure continued therapeutic efficacy against persistent infections - either until no target bacteria remain, or until the residual bacterial population has lost virulence/pathogenicity, for example, due to changes in phage receptors, which often serve essential functions for the bacteria, such as motility. Created with Biorender.com.

*Background R5-5:* **In general, we suggest a phage titer (PFU/mL) of 1e6-9 PFU/mL be applied. If due to the administration route a phage loss is to be expected, we suggest the titer be adapted.**

Reaching the target in the body: The suggested phage titers refer to situations in which efficient delivery of phages to the intended target site can reasonably be assumed, such as superficial wound infections. In many clinical scenarios, however, phage access to the site of infection may be limited due to anatomical barriers including biofilms, or clearance mechanisms such as neutralizing antibodies. Phage stability prior to reaching the target can also be affected by physiological conditions, potentially reducing the effective titer. In addition, inflammatory microenvironment factors, such as pH, proteins, or enzymes, can inactivate phages, and microcirculation and tissue perfusion play a critical role, as poorly perfused tissues may receive lower effective concentrations. In such cases, substantial phage loss may occur before reaching the target, and higher initial titers, e.g., ≥1e10 PFU/mL [132] may be applied to ensure sufficient phage activity at the site of infection. However, data on appropriate phage titers for different infection targets remain very limited.

Route of administration: The route of administration can strongly influence the activity of phages in the human body. For example, a phage loss due to nebulizing, infusing tubing systems or due to gastric acidity should be taken into account. [89,94,127-130]

MOI at the site of infection: The actual phage titer at the site of infection and the resulting MOI may depend on in human conditions of the infection being treated. In many bacterial infections, the initial phage titer should allow for phage replication at the site of infection. This is influenced by the effective MOI at the infection site, which depends on phage and bacterial densities as well as physiological factors. Excessive phage concentrations may trigger inhibitory mechanisms or "lysis from without", potentially disrupting the self-amplifying effect of phage therapy. Conversely, insufficient titers at the site of infection may lead to inadequate bacterial clearance.

Bacterial physiology and population heterogeneity: Furthermore, dormant or stationary-phase bacteria replicate more slowly and are often less susceptible to phage infection. Phages may also have limited activity against intracellular bacteria, further reducing their effectiveness at certain infection sites. Population heterogeneity can influence the efficacy of a given phage titer, as differences in susceptibility exist even within the same bacterial population. Certain bacterial states, such as stress induced by antibiotics, may reduce phage adsorption or replication. Simultaneous antibiotic therapy can either inhibit or enhance phage replication depending on the mechanism, and should be considered when planning the initial dosing.

Finally, depending on the degree of phage neutralization, repeated doses may help offset lower initial titers.

Further key pharmacokinetic and pharmacodynamic considerations for selecting an optimal initial phage dose and route of administration include:

- the concentration of bacterial cells, including both susceptible and resistant populations, along with their respective growth rates
- the numbers of phages present,
- the adsorption or infectivity rate of phages,
- latency periods, and
- burst size, together with the rates of phage-mediated killing, clearance, or inactivation at the infection site [8]

When trying to model the optimal phage titer *in vitro*, for example, through growth kinetics at different MOIs, the focus should be on the patient-specific bacterial strain, as the optimal MOI can vary even among bacteria of the same species. Additionally, it is recommended to determine the phage titer before use from a vial that replicates the conditions of the patient's vial, accounting for potential phage adsorption and titer loss due to (plastic) surface interactions during administration. [130]

These evaluations should be thoroughly discussed in an interdisciplinary team with phage experts. Nevertheless, it must be kept in mind that there is no clear scientific evidence to date in favor of choosing a particular titer and there is a need for further research on this topic.

*Background R5-6 - R5-7:*

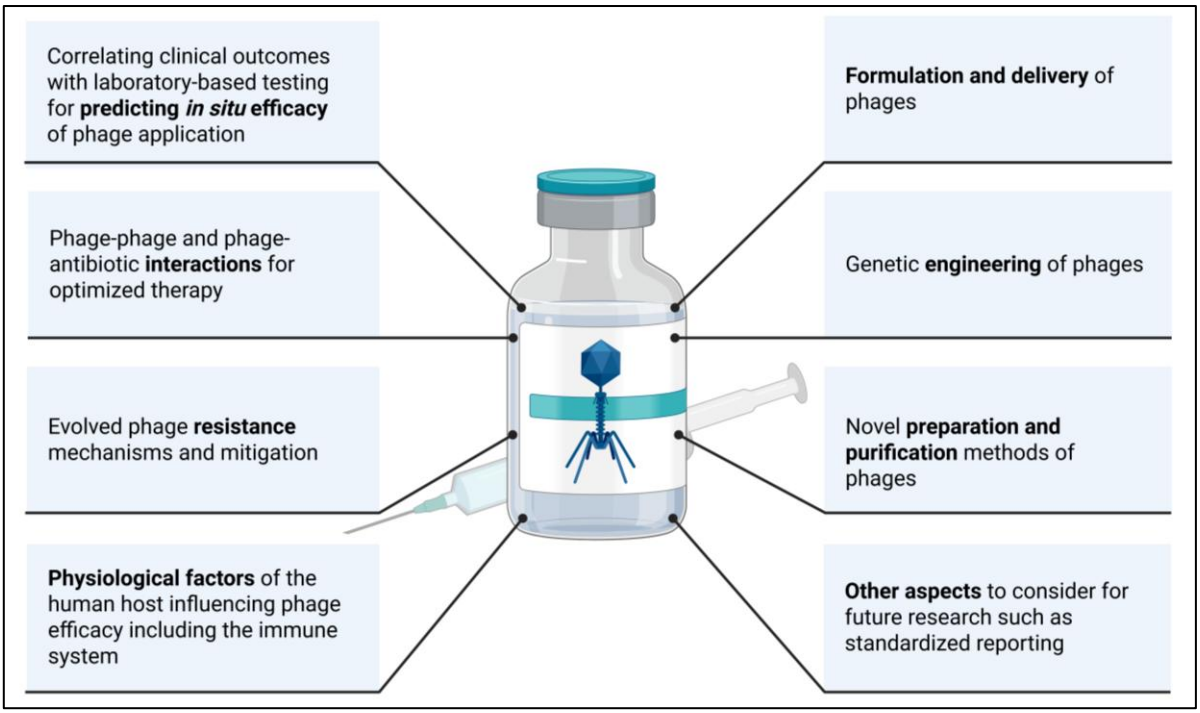
- **A follow-up of at least one year might be considered after phage therapy.**
- **If recurrence of an infection treated with phages is suspected, efforts should be made to confirm or rule out that the recurrence is caused by the same causative pathogen as the initial infection. Results should be documented and referred to a central phage registry if available.**

The duration of follow-up is determined by the clinical context in an interdisciplinary decision-making process involving phage experts, microbiologists, and infectious disease specialists, and could include, for example, monitoring parameters mentioned above in the background text of **R5-3**. Despite sparse data, based on previous experience, a follow-up of 12 months might be considered, if feasible. Ideally, the follow-up period should be extended beyond this period, particularly after treatment of chronic infections. In the overall view, however, the follow-up interval should be harmonized with other treatment concepts for infections.

## Supplementary explanation 5: Recommendations on future research priorities to improve implementation and efficacy of phage therapy

The concept of phage therapy has been known for more than a century. One of the major factors limiting acceptance and successful implementation of phage therapy is the missing evidence from randomized controlled clinical trials proving efficacy of this treatment modality for diverse patient cohorts and indications. However, conducting such clinical trials for every required phage-bacteria combination and indication is not feasible. Still, well-designed studies can serve as exemplary models for other phage-bacteria combinations and indications, playing a crucial role in advancing phage research and establishing its clinical potential. For personalized approaches outside of clinical trials, standardized reporting of outcomes is encouraged. Preferably, outcomes should be reported in a standardized way by participating in international registries such as Phagistry. [78] Moreover, the n-of-1 clinical trial design could serve as a baseline to improve quality in design and reporting of individual patient treatments. [246] The use of artificial intelligence and mathematical modeling is also expected to play a major role for future phage research. Based on available phage host range data, prediction models for phage sensitivity towards different isolates can be developed, reducing the need for extensive phage susceptibility screening in laboratories. [133] Mathematical modeling might also help to approach pharmacokinetic-pharmacodynamic aspects of phage dosing, reducing the amount of necessary *in vitro* or *in vivo* experiments.

To foster the implementation of phage therapy and beyond, important research questions have to be defined and addressed in both settings. This chapter provides an overview of the most important research priorities as defined by this guideline group (**Supplementary explanations figure 7)**. Defined research priorities should serve as guidance for researchers and physicians focusing on translational phage research and designing future projects.




*Supplementary figure 7:* **Research priorities to improve the implementation and efficacy of phage therapy.** Created with Biorender.com.

*Background R6-1 – R6-3:*

**Systematic preclinical studies to investigate the efficacy of phages in the laboratory setting (*in vitro, in vivo)* versus the treatment situation (in reality-near situations) should be conducted.**

**Strategies should be developed that recommend specific approaches (such as testing in biofilm models or *ex vivo, in vivo* models) to increase the probability of high in situ efficacy.**

**Fundamental research (preclinical trials) should be conducted on how phages impact infections caused by more than one pathogen (strain, species, genus) to model phage efficacy in clinical settings.**

To date, only a few published studies have correlated phage therapeutic treatments in patients with prior experimental data. Additionally, in some previous trials, phage susceptibility testing of the target pathogen was not conducted before treatment initiation. [26,28]

In future studies, correlating experimental data with phage susceptibility testing results whenever possible will be crucial for drawing meaningful conclusions about phage efficacy in experimental versus real-life settings. Notably, standard susceptibility testing already reveals differences in phage efficacy between liquid and solid media [134-136], yet it remains unclear which method best reflects human conditions.

Therefore, there is a clear need to standardize susceptibility testing methods across liquid and solid media and to systematically correlate these standardized results with outcomes from clinical administrations. The EUCAST committee on phage susceptibility testing is currently developing a standardized approach to establish a reliable foundation for future evaluations. [77, 159]

Apart from the standard methods currently used for phage susceptibility testing, research should focus on the development and evaluation of innovative approaches for rapid phage diagnostics such as tests based on bioluminescence reactions. [247]

Biofilms are protective layers formed by bacterial communities, making it difficult for antibiotics to penetrate and kill the bacteria inside. In cases of antibiotic-sensitive yet persistent bacterial infections, biofilms can impede the effectiveness of antibiotics, even when the bacteria themselves remain susceptible. However, some phages can break down these biofilms, allowing antibiotics to reach the bacteria more effectively or, in some cases, directly target the bacteria within the biofilm itself. [18,248-251] Comparing the results of standard *in vitro* phage susceptibility testing with phage efficacy and behavior in biofilms presents another significant challenge. Assessing phage activity in biofilms is particularly important for linking predictions to clinical outcomes in relevant treatment scenarios, such as catheter- or implant-associated infections and biofilm eradication in the lungs of cystic fibrosis patients. Therefore, the development of reliable biofilm models should be a focus of future research efforts.

Polymicrobial infections pose a significant challenge for treatment of wounds as well as other types of infections. [31,252,253] Phage research into effective strategies for targeting polymicrobial infections is essential for advancing future administrations. As with monomicrobial infections, studies should focus on assessing phage efficacy in preclinical settings and correlating these findings with outcomes from clinical treatments.

Defining treatment success in phage therapy remains one of the field's most complex and unresolved challenges. Current data are insufficient to support a universally applicable definition. In most clinical scenarios, clinical improvement represents a more realistic and patient-centered endpoint than microbiological eradication, though the latter may be appropriate in selected cases.

*Background R6-4:* **In order to avoid antagonistic phage-phage and phage-antibiotic interactions and to find the optimal combination of antibiotics and phages, in-depth preclinical studies in research consortia should be conducted.**

The phage-phage as well as phage-antibiotics interplay in the therapy context is not yet fully understood and varies significantly depending on the specific phage-phage or phage-antibiotic pairing. The interaction between single phages combined in cocktails, as well as the interaction between phages and different antibiotics, can either be synergistic, additive, neutral, or antagonistic. [31,60,137,138] These interactions can significantly influence therapeutic outcomes, underscoring the need for thorough research and evaluation. Therefore, it is crucial to determine whether specific classes of antibiotics or phage types can be identified as particularly effective with certain phages and host species/isolates, as well as to understand the underlying mechanisms of these interactions. In addition to standardizing phage susceptibility testing criteria for single phages, a standardized method for evaluating phage-phage and phage-antibiotic combinations should also be envisioned in the future. Currently, systematic testing of phage-antibiotic interactions in liquid culture is not performed routinely. However, it should be considered whenever feasible, taking into account antibiogram, phagogram, existing data on the specific phage-antibiotic combination, as well as the time constraints imposed by patient urgency. In alignment with recommendations R6-1 to R6-3, research should focus on investigating and comparing these interactions across various *in vitro* and - if needed and suitable - *in vivo* models, also considering polymicrobial infections.

*Background R6-5 - R6-6:* **Preclinical (*in vitro* and *in vivo*) studies investigating the occurrence of phage resistance should be conducted. These should include studies that (a) elucidate the probability of phage resistance for a particular approach, (b) investigate the relevance of phage resistance to therapy outcomes and identify mitigation strategies, (c) elucidate the exploitation of phage resistance that causes evolutionary costs.**

**In the clinical setting retrospective analyses of samples collected around phage therapy could be exploited to understand evolutionary pathways and to correlate data and findings made during *in vitro* and pre-clinical studies.**

Following phage infection, the regrowth of phage-resistant bacterial variants is frequently observed in preclinical settings. Additionally, numerous clinical reports document the emergence of phage-resistant bacterial variants after phage treatment. [21] Since bacterial regrowth due to the evolution of phage-resistant variants can contribute to persistence of infections and treatment failures, underlying mechanisms for resistance development and mitigation strategies should be key topics of future research. The term evolved phage-resistant variant is preferred over mutant, because phage resistance does not necessarily arise from genomic alterations.

Potential strategies to counteract resistance include the use of high-titer phage lysates, optimized administration processes, phage cocktails, sequential phage administration, or phage-antibiotic combinations. Moreover, evaluating the potential of genetically modified phages in reducing phage resistance development is of particular interest.

Notably, the evolution of phage resistance in bacterial isolates can come at a fitness cost, which can manifest in various ways, such as reduced motility or the reacquisition of antibiotic susceptibility. [118,121,122,124,125] Harnessing these evolutionary costs following phage infection can itself represent a valuable treatment strategy and should thus be equally exploited.

Same as for the recommendations above, phage resistance development should be assessed under different *in vitro* conditions, and - if needed - *in vivo*, and compared to clinical outcomes whenever feasible. To allow in-depth correlation with clinical outcomes, future study design should integrate comprehensive sampling schedules before, during, and following phage treatment.

*Background R6-7 – R6-8:* **Future research should consider the interaction with human physiology, including human cells and immunological aspects.**

**The effect of adverse physiological factors, including, but not limited to, such as extreme pH values, extracellular proteases, and bile acids, reflecting the infection site or relevant conditions, should be investigated to elucidate the pharmacology of phage therapy in the human body.**

When administered to a patient, the pharmacology of therapeutic phages will be influenced by the physiological environment present at the respective infection site. As far as possible, the influence of relevant conditions should be evaluated before treatment initiation, considering the differences in physiological environments between infection sites and the specific phage or phage-antibiotic combinations used. Physiological conditions at the infection sites that may influence phage stability and efficacy are for example low pH (e.g., in the stomach), low oxygenation levels (e.g., in the gut), presence of extracellular proteases or bile acids, or low concentration levels of compounds associated with increased phage efficacy (e.g., calcium or magnesium chloride, free metals [139,140]). The influence of varying conditions in the human body should thus be investigated to optimize future treatment protocols and enhance their efficacy.

Additionally, the role of phage treatment in infections with intracellular pathogen reservoirs (e.g., urinary tract infections with persistent pathogens in the urothelial cells or mycobacteria [254-256]) has to be evaluated, as phage penetration into various human cell types might differ and again be dependent on physiological conditions outlined above.

To support the analysis of factors influencing phage response in the human body, transcriptomic and proteomic analyses of samples collected during treatment might be relevant. [209,257-260]

*Background R6-9:* **To avoid situations during therapy that might negatively impact phage efficacy, preclinical studies (*in vitro* and *in vivo*) should be conducted to examine the interaction of the human body with phages, in particular with the adaptive and innate immune system.**

Due to the lack of clinical sampling around previously conducted phage therapies, insights into phage interaction with the human host and immune system during and following treatment are highly limited. Also, data from experiments analyzing host immune response are scarce. However, it is known that different factors will influence phage pharmacokinetics and efficacy in the human body, with the most relevant ones being:

- Internalization of phages by endocytosis into mammalian cells [141]
- Presence of phage-specific antibodies (pre-existing or evolving following phage administration) that lead to immune neutralization of phages [142,143]
- Presence of phages in the human microbiota that might interact with the therapeutic phages.

Future pre-clinical projects should evaluate these aspects using appropriate models, while future clinical trials should incorporate a sampling strategy allowing the analysis of the above-mentioned aspects as well (e.g., blood samples/peripheral blood mononuclear cells, samples for microbiota analysis).

*Background R6-10:* **Safe and efficient methods for the delivery of therapeutic phages to the site of infection should be developed to ensure stability, including persistence and activity, or to reduce immunogenicity.**

Even if a phage or phage cocktail has been shown to be highly effective against a pathogen in models, the correct phage formulation and delivery method is of importance for successful treatment outcomes. As phages are prone to inactivation by adverse physiological conditions present during storage/delivery but also within the human host as well as immunogenic factors, these have to be taken into account for determining optimal administration strategies. Moreover, physiological conditions appearing during phage storage or administration might affect the stability of the preparation (i.e., buffers used, storage temperature, surface material allowing for phage adherence).

Published case reports and studies describe a large variety of formulation and delivery methods, including topical gels, inhalation, phage-coated intravascular devices, as well as oral and intravenous administration of liquid phage lysates. [21]

For oral administrations, various encapsulation methods have been developed and have proven effective, to ensure efficient delivery of active phages to the infection site. Novel approaches such as encapsulation using nanoparticles, liposomes and nano-emulsions should be exploited to improve future administrations. [91-95,97,144]

In addition to the evaluation of optimized formulation and delivery methods, the timing, co-medication intake, dosing frequency, the need for dynamic adjustments of phage compositions and titers during therapy, and the duration of the therapeutic administration should be further investigated. [18]

*Background R6-11 – R6-12:* **The use of existing molecular biology tools and the development of novel techniques in order to create novel phages with a broader host range and optimized safety profile should be investigated.**

**Pre-clinical studies (*in vitro* and *in vivo*) of bacterial receptor interaction with phage proteins could allow obtaining the knowledge required to create phages with less specificity and thus should be researched.**

Genetic engineering offers large potential for diverse medical treatment strategies including phage therapy. Even though some countries face regulatory hurdles regarding therapeutic administration of genetically modified phages, research should already focus on tackling shortcomings associated with phage therapy by molecular biology tools.

It has been demonstrated that one major disadvantage of phage therapy – the limited host range of phages towards different isolates within a species or between different species – can be overcome by modification of receptor binding proteins or via modification of encoded proteins assisting in bacterial cell infection. By removing lysogeny genes such as integrases, the potential of temperate phages that are currently not considered for treatment can be exploited if no lytic phages are available for a specific patient case. [63]

The basis for this targeted engineering of phages is the thorough pre-clinical evaluation of basic phage biology in different models; however, wherever possible these should be correlated to real-life data derived from patient treatment.

*Background R6-13:* **To accelerate the preparation and downstream processing of therapeutic phage solutions, research should be conducted to develop rapid protocols and/or methods that allow the efficient purification of phages, and the concomitant removal of undesirable substances such as endotoxins.**

Currently, a limited number of methods for removing impurities, such as endotoxins, are available, many of which are time-consuming, expensive and/or result in a significant loss of phage titer. Novel solutions for efficient preparation, such as the cell-free preparation of phages [145-147,152], and further purification methods of phages should thus be developed.

# List of Supplementary explanations - tables



# List of Supplementary figures



# Abbreviations

Abbreviations of involved societies, authorities, and institutions can be found in **Supplementary methods - table 1** "Involved professional societies and experts".

| Acronym | Full term |
|---|---|
| AMG | German Medicines Act |
| AMR | Antimicrobial resistance |
| AMWHV | Medicines and Active Substances Manufacturing Ordinance |
| API | Active pharmaceutical ingredient |
| ApBetrO | German Pharmaceutical Operations Regulation |
| ASTM | ASTM International, formerly American Society for Testing and Materials |
| BAK | Federal Chamber of Pharmacists |
| BSL | Biosafety level |
| COI | Conflict of interest |
| CPMP-ICH | Committee for Proprietary Medicinal Products – International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| DAO | Double agar overlay |
| DSP | Downstream processing |
| ddPCR | Droplet digital PCR |

| Acronym | Full term |
|---|---|
| EDQM | European Directorate for the Quality of Medicines & Healthcare |
| ELISA | Enzyme-linked immunosorbent assay |
| EMA | European Medicines Agency |
| EOP | Efficiency of plating |
| EP (method) | European Pharmacopoeia (method) |
| ESCMID | European Society of Clinical Microbiology and Infectious Diseases (ESCMID) |
| ESGNTA | Study Group for Non-Traditional Antibacterial Therapy of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) |
| EU | European Union |
| EU/kg | Endotoxin units/kilogram |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| GMP | Good Manufacturing Practice |
| ICH | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use |
| IMP | Investigational medicinal product |
| i.v. | intravenous |
| LAL | Limulus amebocyte lysate |
| MAT | Monocyte activation test |
| MCB | Master cell bank |
| MOI | Multiplicity of infection |
| MPSL | Master phage seed lot |
| MTA | Material transfer agreement |
| NIH | National Institutes of Health |
| PFU | Plaque-forming units |
| Phage | Bacteriophage |
| Ph.Eur. | European Pharmacopoeia |
| PICC | Peripherally inserted central catheter |
| PTMP | Phage therapy medicinal product |
| RABS | Restricted access barriers systems |
| RCT | Randomized controlled trial |

| Acronym | Full term |
|---|---|
| rFC | Recombinant factor C test |
| RiliBÄK | Guideline of the German Medical Association |
| RPSL | Research phage seed lot |
| SDS-PAGE | Sodium dodecyl sulfate polyacrylamide gel electrophoresis |
| TAB | Office of Technology Assessment at the German Bundestag |
| TSE | Transmissible spongiform encephalopathy |
| TXTL | Transcription/translation systems |
| USP | Upstream processing |
| UV | Ultraviolet |
| WCB | Working cell bank |
| WG | Working group |
| WPSL | Working phage seed lot |
| WTA | Wall teichoic acid |

# A Consensus-based Guideline for Personalized Bacteriophage Therapy

# Supplementary methods

# Details on the guideline methodology

## Disclosure of interests and management of conflicts

In accordance with the guidelines of the Association of the Scientific Medical Societies in Germany (AWMF) on managing conflicts of interest, all participants submitted their declarations using the corresponding AWMF form on the online AWMF conflict of interest (COI) portal (see **Supplementary file 1**). The COI entries were reviewed by the steering committee of the guideline and by a neutral reviewer and categorized according to the AWMF criteria as no, minor, moderate, or strong in relation to the individual provisions. The neutral reviewer was equally evaluated by a second neutral individual. Both individuals were not formally part of the guideline group but listed in the AWMF COI portal (**Supplementary file 1**).

**No COIs:** The management of research projects or the conduct of clinical studies exclusively publicly funded were not regarded as COI. Additionally, advisory board activities unrelated to phages were not classified as conflicts of interest.

**Minor COIs:** Paid lecture and/or training activities, compensated consulting for a scientific journal, or editorial work for a scientific journal, as well as paid authorship or co-authorship related to the guideline topic, were considered minor COIs. Personal relationships (as a partner or first-degree relative) with authorized representatives of a healthcare company connected to phages were also classified as minor conflicts of interest. As a result, minor conflicts of interest were found for three guideline members. Minor conflicts of interest led to the appointment of a co-coordinator without thematically relevant conflicts of interest. Minor conflicts of interest had no consequences in relation to the voting results.

**Moderate COIs:**

- Consulting or expert services, regular lecturing activities, or paid work in a scientific advisory board of a company in the healthcare industry (e.g., pharmaceutical industry, medical device industry), a commercially oriented contract research organization, or an insurance company related to phages.
- Research projects/conduct of clinical studies: financial contributions (third-party funding) for research projects or direct funding of staff from a company in the healthcare sector, a commercially oriented contract research organization, or an insurance company related to phages. Only management responsibility was classified as moderate conflict of interest.
- Share ownership of individual companies related to phages.
- Leadership in training and continuing education with direct industry funding by companies related to phages.

As a result, moderate conflicts of interest were found for seven guideline members (**Supplementary file 1**). Moderate conflicts of interest led to double counting of the votes (1 × without, 1 × with the affected persons). Moderate conflicts did not affect the strength of consensus for any of the recommendations or statements in this guideline. Moreover, moderate conflicts of interest necessitated exclusion from leadership and coordination responsibilities.

**Strong COIs:** Owner interests (patents, copyrights, significant ownership of shares, stocks, or funds involving companies in the phage sector) and employment in the industrial sector were classified as strong conflicts of interest. No strong conflicts of interest related to the guideline were identified.

Protective factors against bias include the interdisciplinary, representative composition of the guideline group as well as the structured consensus-building under neutral moderation.

## Supplementary methods - table 1: Involved professional societies and experts

The following table details involved professional societies and experts. [a, b]

*Supplementary methods - table 1:* Involved professional societies and experts

| | **Mandate holder/ Deputy/Expert** | **First name** | **Last name** |
|---|---|---|---|
| **Societies** | | | |
| ADKA: Federal Association of German Hospital Pharmacists (*Bundesverband Deutscher Krankenhausapotheker*) | Mandate holder | Oliver | Hartwich |
| ADKA: Federal Association of German Hospital Pharmacists (*Bundesverband Deutscher Krankenhausapotheker*) | Deputy | Melanie | Kempe |
| DDG: German Dermatological Society (*Deutsche Dermatologische Gesellschaft*) | Mandate holder | Manuel | Cornely |
| DDG: German Dermatological Society (*Deutsche Dermatologische Gesellschaft*) | Deputy | Diana | Rajewski |
| DGG: German Society for Vascular Surgery and Medicine (*Deutsche Gesellschaft für Gefäßchirurgie und Gefäßmedizin*) | Mandate holder | Justus | Groß |
| DGHM: German Society for Hygiene and Microbiology (*Deutsche Gesellschaft für Hygiene und Mikrobiologie*) | Mandate holder | Evgeny | Idelevich |
| DGHNO-KC: German Society for Otorhinolaryngology, Head and Neck Surgery (*Deutsche Gesellschaft für Hals-Nasen-Ohren-Heilkunde, Kopf- und Hals-Chirurgie*) | Mandate holder | Sarina | Müller |
| DGI: German Society for Infectious Diseases (*Deutsche Gesellschaft für Infektiologie*) | Mandate holder | Simone C. | Lieberknecht-Jouy |

[a] Li Deng was initially part of the guideline group but, due to strong conflicts of interest, was made available for consultation by AWMF instead.

[b] The mandate holder and deputy of the German Society for Thoracic, Cardiac, and Vascular Surgery (DGTHG) did not submit their declarations of interest (COIs) in the AWMF portal, which is why they could not be involved in drafting this guideline despite initial registration.

| | **Mandate holder/ Deputy/Expert** | **First name** | **Last name** |
|---|---|---|---|
| DGI: German Society for Infectious Diseases (*Deutsche Gesellschaft für Infektiologie*) | Deputy | Ulrich | Matt |
| DGIM: German Society of Internal Medicine (*Deutsche Gesellschaft für Innere Medizin*) | Mandate holder | Silvia | Würstle |
| DGIM: German Society of Internal Medicine (*Deutsche Gesellschaft für Innere Medizin*) | Deputy | Annika Y. | Classen |
| DGKJ: German Society for Pediatrics and Adolescent Medicine (*Deutsche Gesellschaft für Kinder- und Jugendmedizin*) | Mandate holder | Renate | Krüger |
| DGOU: German Society for Orthopedics and Trauma Surgery (*Deutsche Gesellschaft für Orthopädie und Unfallchirurgie*) | Deputy | Markus | Rupp |
| DGOU: German Society for Orthopedics and Trauma Surgery (*Deutsche Gesellschaft für Orthopädie und Unfallchirurgie*) | Mandate holder | Volker | Alt |
| DGP: German Respiratory Society (*Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin*) | Mandate holder | Martin | Witzenrath |
| DGP: German Respiratory Society (*Deutsche Gesellschaft für Pneumologie und Beatmungsmedizin*) | Deputy | Mirjam | Stahl |
| DGPI: German Society for Pediatric Infectiology (*Deutsche Gesellschaft für Pädiatrische Infektiologie*) | Mandate holder | Rebecca | Knoll |
| DGPI: German Society for Pediatric Infectiology (*Deutsche Gesellschaft für Pädiatrische Infektiologie*) | Deputy | Johannes | Hübner |
| DGVS: German Society for Gastroenterology, Digestive, and Metabolic Diseases (*Deutsche Gesellschaft für Gastroenterologie, Verdauungs- und Stoffwechselkrankheiten*) | Mandate holder | Münevver | Demir |
| DGWMP: German Society for Military Medicine and Pharmacy (*Deutsche Gesellschaft für Wehrmedizin und Wehrpharmazie*) | Mandate holder | Christian | Willy |
| DGWMP: German Society for Military Medicine and Pharmacy | Deputy | Joachim | Bugert |

| | **Mandate holder/ Deputy/Expert** | **First name** | **Last name** |
|---|---|---|---|
| (*Deutsche Gesellschaft für Wehrmedizin und Wehrpharmazie*) | | | |
| DGZMK: German Society for Dental, Oral, and Maxillofacial Medicine (*Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde*) | Mandate holder | Julia | Heider |
| DZIF: German Center for Infection Research (*Deutsches Zentrum für Infektionsforschung*) | Mandate holder | Annika Y. | Classen |
| DZIF: German Center for Infection Research (*Deutsches Zentrum für Infektionsforschung*) | Deputy | Maria J. G. T. | Vehreschild |
| GfV: Society for Virology (*Gesellschaft für Virologie*) | Mandate holder | Helmut | Fickenscher |
| PEG: Paul Ehrlich Society for Infection Therapy (*Paul-Ehrlich-Gesellschaft für Infektionstherapie*) | Mandate holder | Mathias W. | Pletz |
| VAAM: Association for General and Applied Microbiology (*Vereinigung für Allgemeine und Angewandte Mikrobiologie*) | Mandate holder | Stefanie | Barbirz |
| VAAM: Association for General and Applied Microbiology (*Vereinigung für Allgemeine und Angewandte Mikrobiologie*) | Deputy | Tessa | Quax |
| **Experts - Authorities and Institutions** | | | |
| BfArM: Federal Institute for Drugs and Medical Devices, German | Expert | Anja | Düchting[c] |
| BfR: Federal Institute for Risk Assessment, Germany | Expert | Jens | Hammerl |
| Center for Phage Biology and Therapy at Yale University, USA | Expert | Paul | Turner |
| Center for Phage Biology and Therapy at Yale University, USA | Expert | Benjamin | Chan |
| University Hospital Center of Lyon, France | Expert | Tristan | Ferry |
| DSMZ: Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Germany | Expert | Christine | Rohde |
| DSMZ: Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Germany | Expert | Johannes | Wittmann |
| Fraunhofer Institute for Toxicology and Experimental Medicine, Germany | Expert | Holger | Ziehr |

| | **Mandate holder/ Deputy/Expert** | **First name** | **Last name** |
|---|---|---|---|
| Fraunhofer Institute for Toxicology and Experimental Medicine, Germany | Expert | Imke | Korf |
| HMU: Health and Medical University, Germany | Expert | Sebastian | Leptihn |
| PEI: Paul-Ehrlich-Institute, Germany | Expert | Holger | Lößner[c] |
| PEI: Paul-Ehrlich-Institute, Germany | Expert | Oleg | Krut[c] |
| QAMH: Queen-Astrid-Military-Hospital, Belgium | Expert | Jean-Paul | Pirnay |
| Sciensano (National Institute for Health and Environment), Belgium | Expert | Pieter-Jan | Ceyssens |
| Sciensano (National Institute for Health and Environment), Belgium | Expert | Mathieu | de Jode |
| University Clinic Balgrist, Switzerland | Expert | Shawna | McCallin |
| University Clinic Balgrist, Switzerland | Expert | Lorenz | Leitner |
| Veterinary medicine, National Forum for Phages, Germany | Expert | Wolfgang | Beyer |

# Supplementary methods - table 2: Patient representatives

*Supplementary methods - table 2:* Patient representatives

| **Society** | **Mandate holder/ Deputy** | **First name** | **Last name** |
|---|---|---|---|
| BDO: Federal Association of Organ Transplant Recipients (*Bundesverband der Organtransplantierten*) | Mandate holder | Matthias | Maelteni |
| BDO: Federal Association of Organ Transplant Recipients (*Bundesverband der Organtransplantierten*) | Deputy | Sandra | Zumpfe |
| Federal Association for Cystic Fibrosis (*Bundesverband Cystische Fibrose*) | Mandate holder | Sylvia | Hafkemeyer |
| Federal Association for Cystic Fibrosis (*Bundesverband Cystische Fibrose*) | Deputy | Jutta | Bend |

[c] The views expressed in this article are the personal views of the author(s) and may not be understood or quoted as being made on behalf of or reflecting the position of the regulatory agency/agencies or organizations with which the author(s) is/are employed/affiliated.

# Supplementary methods - table 3: Steering committee

*Supplementary methods - table 3:* Steering committee

| Societies | First name | Last name |
|---|---|---|
| DGIM: German Society of Internal Medicine (*Deutsche Gesellschaft für Innere Medizin*) | Silvia | Würstle |
| DGI: German Society for Infectious Diseases e.V. (*Deutsche Gesellschaft für Infektiologie*) | Simone C. | Lieberknecht-Jouy |
| DZIF: German Center for Infection Research e.V. (*Deutsches Zentrum für Infektionsforschung*) | Maria J. G. T. | Vehreschild |
| DZIF: German Center for Infection Research e.V. (*Deutsches Zentrum für Infektionsforschung*) | Annika Y. | Classen |

# Supplementary methods - table 4: Working groups

*Supplementary methods - table 4:* Working groups

| Working group number | Working group title | Group leaders |
|---|---|---|
| 1 | Guideline development and organization | Steering committee: Silvia Würstle, Simone C. Lieberknecht-Jouy, Maria J.G.T. Vehreschild, Annika Y. Classen |
| 2 | General principles of personalized phage therapy | Christian Willy |
| 3 | Infrastructure of personalized phage therapy | Shawna McCallin |
| 4 | Preparation and quality control of personalized phage therapeutics | Simone C. Lieberknecht-Jouy |
| 5 | Administration of personalized phage therapy | Christian Willy |
| 6 | Research priorities to improve implementation and efficacy of phage therapy | Sebastian Leptihn |

# Literature search and structured consensus meetings

During an initial meeting (kick-off meeting), the content and methodological approaches were established, including key questions for each defined working group (WG) and the literature search strategy. Subsequently, each WG conducted an individual literature search and consolidated expertise and practical insights. Based on the identified literature and expert suggestions, the recommendations and background texts were developed by the WG and initially reviewed within the whole WG through

an email circulation process managed by the WG leaders. The grading of the recommendations was based on the terms indicated in **Supplementary methods - table 5**.

*Supplementary methods - table 5:* Grading – strength of the recommendation

| **Grading – strength of the recommendation** | | |
|---|---|---|
| **Description** | **Syntax** | **Abbreviation** |
| Strong recommendation | - We recommend/we do not recommend<br>- Should/should not | A |
| Conditional recommendation | - We suggest/we do not suggest/we suggest not<br>- Ought to/ought not to<br>- Can/cannot | B |
| Open recommendation | - May be (considered)/May be (omitted)<br>- Could/could not be (considered)<br>- Might/might not be (considered) | Optional |

Following a circulation of the recommendations within the whole guideline group, all recommendations were discussed among the guideline participants neutrally moderated by an AWMF representative during six structured online consensus meetings. The recommendations were voted on according to the principles of the NIH (National Institutes of Health) conference: presentation in the full plenary considering comments and clarifications by the WG leaders, inclusion of statements, possible amendments, voting, and finalizing the results. Comments and suggested changes from the discussion were reviewed by the WGs and steering committee. Recommendations were revised accordingly and re-submitted for voting where required. The strength of the consensus was determined according to **Supplementary methods - table 6**.

*Supplementary methods - table 6:* Classification of strength of consensus

| **Classification of strength of consensus** | |
|---|---|
| **Consensus** | **% approval** |
| Strong consensus | > 95% of the participants |
| Consensus | > 75 - 95 % of the participants |
| Majority approval | > 50 - 75 % of the participants |
| No consensus | ≤ 50 % of the participants |

Results from the literature searches regarding the background text from the individual WGs were supplemented by the steering committee. The entire guideline group and specifically designated reviewers were given the opportunity to review the text and suggest additional references or adjust the existing ones.

# Voting procedure

The voting on recommendations during the consensus conferences was conducted online via the integrated survey tool of the videoconference platform (Zoom Communications Inc.). Additionally, the voting on the definitions proposed by WG 4 was carried out using the online platform *SurveyMonkey (https://de.surveymonkey.com/* [261]*)*, while the voting on **Supplementary explanations - table 2** of WG 4 was performed through the online tool *Terminplaner (https://terminplaner6.dfn.de/* [262]*).*

For each vote, the following options were provided to allow differentiation with regard to potential conflicts of interest:

Do you agree with the recommendation?

Mandate holder/deputy - no/minor COI (conflict of interest):
- ☐ Agree
- ☐ Do not agree
- ☐ Abstention

Mandate holder/deputy - moderate COI:
- ☐ Agree
- ☐ Do not agree
- ☐ Abstention

Expert - no/minor COI:
- ☐ Agree
- ☐ Do not agree
- ☐ Abstention

Expert - moderate COI:
- ☐ Agree
- ☐ Do not agree
- ☐ Abstention

# Supplementary methods - table 7: External reviewers

*Supplementary methods - table 7:* External reviewers

| Affiliation | Involvement | Field of expertise | First name | Last name |
|---|---|---|---|---|
| Martin-Luther-University Halle-Wittenberg, Germany | External reviewer | Legal/Regulatory | Timo | Faltus |
| Federal Union of German Associations of Pharmacists (*Bundesvereinigung Deutscher* | External reviewer | Pharmaceutics | Peter | Ruth |

| Affiliation | Involvement | Field of expertise | First name | Last name |
|---|---|---|---|---|
| *Apothekerverbände*), Germany | | | | |
| Frankfurt Foundation Quality of Medicines, Germany | External reviewer | Quality assurance | Henning | Blume |
| Becky Mayer Centre for Phage Research, University of Leicester, UK | External reviewer | Translational phage research and application | Martha | Clokie |
| Kyambogo University, Kampala, Uganda | External reviewer | Translational phage research and application | Ritah | Nakayinga |
| Pusan National University School of Medicine, South Korea | External reviewer | Translational phage research and application | Shinwon | Lee |
| Hadassah Hebrew University Medical Center, Israel | External reviewer | Translational phage research and application | Ran | Nir-Paz |
| Mayo Clinic, Rochester, USA | External reviewer | Translational phage research and application | Gina | Suh |
| University of Sydney, Australia | External reviewer | Translational phage research and application | Jon | Iredell |
| University of Toronto, Canada | External reviewer | Translational phage research and application | Gregory | German |

## Supplementary methods - table 8: Endorsing societies and organizations

*Supplementary methods - table 8:* Societies and organizations endorsing the recommendations provided within this guideline. The endorsement process depended on the individual procedures and requirements of the respective societies or organizations.

| Society/-Organization | Representative | Country/Region | Status |
|---|---|---|---|
| Phage Directory | Jessica Sacher (Phage Directory, Co-founder) | International | Endorsed |
| PhageAfrica (Africa Phage Forum) | Emmanuel Nnadi | Africa | Endorsed |
| Infectious Diseases Society of America (IDSA) | IDSA Practice Guidelines Team | USA | Endorsed |

| Society/-Organization | Representative | Country/Region | Status |
|---|---|---|---|
| Japanese Association for Infectious Diseases (JAID) | Tetsuya Matsumoto (JAID president) | Japan | Endorsed |
| Latvian antimicrobial resistance competence center | Uga Dumpis (Head of Latvian National AMR competence center) | Latvia | Endorsed |
| Study Group for Non-Traditional Antibacterial Therapy of the European Society of Clinical Microbiology (ESGNTA, ESCMID) | Ran Nir-Paz (ESGNTA, Chair executive committee) | Europe | Endorsed |
| European Committee on Antimicrobial Susceptibility Testing (EUCAST), Phage Susceptibility Testing Subcommittee | Frederic Laurent (Chair scientific steering committee) | Europe | Endorsed* |
| European Society for Paediatric Infectious Diseases (ESPID) | Adilia Warris (ESPID President) | Europe | Endorsed |
| Australasian Society for Infectious Diseases (ASID) | Irene Kourtis (ASID Chief executive officer) | Australasia | Endorsed |

*The EUCAST Phage Susceptibility Testing Subcommittee, in line with its specific area of expertise, endorses the recommendations on phage susceptibility testing and expresses overall support for this guideline.

## Validity period and update procedure

The validity of this guideline was confirmed by the mandate holders of the participating professional societies, working groups, organizations, and associations in June 2025 and was thereby approved in its entirety. This guideline is valid from January 5, 2026, through to June 29, 2030. The revision will be initiated by the steering committee. Additionally, the steering committee will review the need for updates to the guideline annually. For further information, the DGI office (administration@dgi-net.de) is available as a contact.

# List of Supplementary methods – tables

# A Consensus-based Guideline for Personalized Bacteriophage Therapy

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# Supplementary File 1 - Summary of the declarations of conflicts of interest

The following presents a tabular summary of the declarations of interests along with the results of the conflicts of interest assessment and the measures adopted after discussion of the facts by the guideline development group and implemented during the consensus conference.

**Guideline coordination: Würstle, Silvia; Lieberknecht-Jouy, Simone C.; Vehreschild, Maria J. G. T.; Classen, Annika.**

**Guideline: Personalized Bacteriophage Therapy**
**AWMF Registry number: 092 – 003, Version 1.0**

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| Alt, Volker | Bio-Gate | No | No | No | No | No | Member: Chair, Musculoskeletal Infections Section, German Society for Orthopaedics and Trauma Surgery | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |
| Classen, Annika Yanina | No | No | Goethe University Frankfurt, Academy for Infectious Medicine., Roland Berger | No | German Center for Infection Research e.V. (DZIF e.V.) | No | Member: Professional associations: German Society for Infectious Diseases (DGI), German Society for Internal Medicine (DGIM), German Society for Hematology and Medical Oncology/ Infectious Diseases Working Party (DGHO/AGIHO), European Society of Clinical Microbiology and Infectious Diseases/ Study Group for Non-Traditional Antimicrobial Therapies (ESCMID/ESGNTA), Guideline contributions: DGHO/AGIHO, scientific activities: Antibiotic resistance development, microbiome research, phage therapy, clinical activities: Clinic I for Internal Medicine (Hematology/Oncology and | Topics relevant to phage research and therapy<br><br>COI minor: limitation of management (coordination) functions [1] |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Infectious Diseases), University Hospital Cologne, participation in education/training: Organized the first DZIF Symposium on “Translational Phage Research and Application in Germany,” July 2022, personal relationships: None | |
| Barbirz, Stefanie | No | No | No | No | German Research Foundation (DFG) | No | Member: Association for General and Applied Microbiology (VAAM), Society for Biochemistry and Molecular Biology (GBM), German Association for Synthetic Biology (GASB), German Chemical Society (GdCh) | No COI |
| Bend, Jutta | No | No | No | No | No | No | Member: Association for General and Applied Microbiology (VAAM), German Network for Evidence-Based Medicine (DNebm), European Cystic Fibrosis Society (ECFS) | No COI |
| Beyer, Wolfgang | Laboklin Bad Kissingen | No | No | No | No | No | Scientific activity: research and teaching in veterinary microbiology and immunology at the University of Hohenheim, scientific lead of the 1st German Phage Symposium, Hohenheim, 2017, scientific lead of the 2nd German Phage Symposium, Hohenheim, 2022 | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |
| Blume, Henning (Reviewer) | No | No | No | No | No | No | Member: numerous, predominantly international societies of pharmaceutical sciences | No COI |
| Bugert, Joachim Jakob | Virus Genes | EDF Resilience PHAGE | No | No | No | No | Member: German Society for Virology (GfV), American Society for Virology (ASV) | Topics relevant to phage research and therapy<br><br>COI minor: limitation of management (coordination) functions [1] |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| Ceyssens, Pieter-Jan | No | No | No | No | No | No | Member: Belgian Society Viruses of Microbes (BSVoM), board member, Scientific activity: Responsible for quality control of therapeutic phages in Belgium, for magistral application, Participation in education/training: Chair Bacteriophage working party at EDQM | No COI |
| Chan, Benjamin | No | No | No | No | No | No | No | No COI |
| Cornely, Manuel | No | No | No | No | No | No | Member: Delegate of the German Society of Dermatology (DDG), Scientific activity: Delegate of the DDG, Clinical activity: Delegate of the DDG, Participation in education/training: Delegate of the DDG, Personal relationships: Delegate of the DDG | No COI |
| Demir, Münevver | No | Gilead Sciences | AbbVie Deutschland, Ipsen, Gilead | No | No | No | Member: German Society for Gastroenterology, Digestive and Metabolic Diseases (DGVS), member of the German Society for Internal Medicine (DGIM), member of the European Association for the Study of the Liver (EASL), scientific activity: liver, microbiome, gastroenterology, clinical activity: hepatology, gastroenterology, liver transplantation, internal medicine, participation in education/training: none, personal relationships: none | No COI |
| Düchting, Anja | No | No | No | No | No | No | No | No COI |
| Faltus, Timo (Reviewer) | Editorial board of Bundesgesundheitsblatt | No | No | Editorial board of Bundesgesundheitsblatt | BMBF | No | Scientific activity: Legal handling of phage therapy | No COI |
| Ferry, Tristan | ARMATA | Pherecydes | No | No | No | No | Member: European Society of | Topics relevant to phage |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Clinical Microbiology and Infectious Diseases/ Study Group for Non-Traditional Antimicrobial Therapies (ESCMID/ESGNTA) | research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |
| Fickenscher, Helmut | Land SH | RKI, Land SH | No | No | Third-party funding from state and federal sources | No | Member: German Society for Hygiene and Microbiology (DGHM), board member; German Society for Virology (GfV), advisory board member; German Society for Virology and Vaccinology (DVV), interim president; Bavarian Association of Microbiologists and Infection Specialists (BÄMI), state chair, scientific activity: infection epidemiology, molecular and clinical virology and microbiology, isolation and function of bacteriophages, clinical activity: microbiological diagnostics, participation in education/training: none, personal relationships: none | No COI |
| Groß, Justus | No | No | No | No | No | No | Member: none, Personal relationships: CEO Medical Director phaTEC- GmbH | Topics relevant to phage research and therapy<br><br>COI minor: limitation of management (coordination) functions [1] |
| Hafkemeyer, Sylvia | No | No | No | No | No | No | Member: European Cystic Fibrosis Society (ECFS) | No COI |
| Hammerl, Jens Andre | Reviewer of scientific publications and research proposals | No | Presenter of scientific contributions in the field of research | No | Applicant for research projects in the fields of diagnostics, antimicrobial resistance, and phages | No | Scientific activity: detection and characterization of food-associated bacteria (e.g., Yersinia, Vibrio, E. coli, Klebsiella), studies on antimicrobial resistance dissemination, transmission routes, and horizontal gene transfer, | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | detection and characterization of phages against, e.g., MRSA, Pseudomonas, Klebsiella, Yersinia, assessment of phage suitability for applications, participation in education/training: organization of a student course on whole-genome sequencing, organization of a student course on phage isolation and characterization | |
| Hartwich, Oliver | No | No | No | No | No | No | Member: German Association of Hospital Pharmacists (ADKA e.V.), clinical activity: oncology pharmacy, clinical trials, antibiotic stewardship | No COI |
| Heider, Julia | No | No | No | No | No | No | Scientific activity: microbiology, immunology, dysgnathia, cleft lip and palate (CLP), maxillofacial surgery, clinical activity: dysgnathia, CLP, craniofacial malformations, participation in education/training: dental students, medical students, midwives, nursing students, speech therapists, personal relationships: none | No COI |
| Hübner, Johannes | GSK, MSD, BioNTech, AstraZeneca | Gilead, MSD, GSK, Shionogi | No | MSD | No | No | Member: German Society for Pediatric Infectious Diseases (DGPI) | No COI |
| Idelevich, Evgeny | IQTIG | No | MSD, Bruker | No | Federal Ministry of Education and Research (BMBF), MetaSystems Hard & Software GmbH, State of Mecklenburg-Western Pomerania (ERDF), Federal Ministry of Education and Research (BMBF) | University of Münster / Bruker | Member: German Society for Hygiene and Microbiology (DGHM), German Society for Mycology (DMykG), European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Paul Ehrlich Group (PEG), scientific activity: sepsis diagnostics, diagnostics and treatment of infections caused by MDR microorganisms, clinical activity: microbiological diagnostics, | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | antibiotic consultation | |
| Kempe, Melanie | Academy of Infectious Medicine | Medical Association of North Rhine | No | No | No | No | Member: German Association of Hospital Pharmacists (ADKA e.V.) , | No COI |
| Knoll, Rebecca | No | No | No | No | Vertex Pharmaceuticals | No | Member: German Society for Pediatric Infectious Diseases (DGPI), German Society for Pediatrics and Adolescent Medicine (DGKJ), scientific activity: cystic fibrosis, microbiome, clinical activity: general pediatrics, pediatric intensive care, pneumology, participation in education/training: co-organization of the training series Paed-ID Meet the Expert of the Young DGPI | No COI |
| Kopp, Ina (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) | Not applicable (AWMF coordinator) |
| Korf, Imke | No | No | No | No | Federal Ministry of Education and Research (BMBF) and The Federal Joint Committee GB-A | No | Clinical activity: production of phages as biologics | No COI |
| Krut, Oleg | No | No | No | No | No | No | No | No COI |
| Krüger, Renate | No | No | No | No | No | No | No | No COI |
| Leitner, Lorenz | No | No | No | No | No | No | No | No COI |
| Leptihn, Sebastian | No | No | No | No | No | No | Member: DZIF TransPhage Net | No COI |
| Lieberknecht-Jouy, Simone C. | No | No | No | No | No | No | Member: German Society for Infectious Diseases (DGI), scientific activity: research on the microbiome and phages, clinical activity: | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Department of Infectious Diseases | |
| Lößner, Holger | No | No | No | No | No | No | Member: German Society for Hygiene and Microbiology (DGHM), working group “Microbial Viruses,” scientific activity: Kinetic Fingerprinting Links Bacteria-Phage Interactions with Emergent Dynamics: Rapid Depletion of Klebsiella pneumoniae Indicates Phage Synergy. | No COI |
| Maelteni, Matthias | No | No | No | No | No | No | Member: Federal Association of Organ Transplant Recipients (BDO e.V.), initially deputy regional group leader (deputy RGL) Munich, later regional group leader Munich and deputy chairperson of the BDO e.V. | No COI |
| Matt, Ulrich | No | No | State Medical Association of Hesse | No | No | No | Member: Medical Association of Hesse, American Thoracic Society, Austrian Society for Infectious Diseases and Tropical Medicine, scientific activity: functional properties of macrophages during infections, scientific activity: rat-bite fever, clinical activity: clinical infectious diseases | No COI |
| McCallin, Shawna | APT, Phages4A | GSK | Phages for Global Health | No | No | No | Member: International Society of Viruses of Microbes (ISVM), executive board secretary, European Society of Clinical Microbiology and Infectious Diseases/ Study Group for Non-Traditional Antimicrobial Therapies (ESCMID/ESGNTA), executive board secretary, scientific activity: Phage therapy, clinical activity: Clinical trials for phage therapy, participation in education/training: Phages for Global | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Health, course instructor | |
| Müller, Sarina | No | No | No | No | No | No | No | No COI |
| Nothacker, Monika (AWMF coordinator) | No paid activities | - Health services research project INDiQ (Measurement of indication quality from routine data – compensation as stated), Steering Committee of the National Cancer Plan – no compensation, IQTIG | Berlin School of Public Health | No | German Cancer Aid , Network University Medicine COVID-19, BMG, Network University Medicine for Pandemic Preparedness 2.0, G-BA Innovation Fund | No | Member: - German Network Evidence Based Medicine<br>- German Cancer Society (until 12/2020)<br>- Guidelines International Network/GRADE Working Group (member), Scientific activity: Guidelines and Guideline Methodology, Methodology of guidelines based performance measures/quality indicators, Clinical activity: no clinical activity or clinical research, Participation in education/training: Guideline seminars within Curriculum for guideline developers in Germany , Personal relationships: no | Not applicable (AWMF coordinator) |
| Pirnay, Jean-Paul | GlaxoSmithKline Biologicals SA | No | No | No | Walloon Government | No | Member: P-H-A-G-E.org (http://www.p-h-a-g-e.org/en/), Member: European Society of Clinical Microbiology and Infectious Diseases/ Study Group for Non-Traditional Antimicrobial Therapies (ESCMID/ESGNTAESCMID, Member: Belgian Superior Health Council, Member: European Medicines Agency (EMA): NTWP Operational Expert Group on Bacteriophages, Member: Member of the Belgian EMBL (European Molecular Biology Laboratory) accompanying committee, Member: Appointed by the European Commission as expert and Vice-Chair Cross Reader in the evaluation of research proposals in relation to the Seventh Framework (FP7), | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Horizon 2020, and Horizon Europe programs of the European Commission, Member: NATO Research and Technology Organization. Team leader (Belgium is lead nation) of Research Task Group RTG-313 on the “Introduction of phage therapy in military medicine”, Member: Partner in Phages for Global Health (http://www.phagesforglobalhealth.org) | |
| Pletz, Mathias | No | MSD, Pfizer, Shionogi, Roche, Sanofi, Janssen, AstraZeneca | MSD, Pfizer, Shionogi, Bayer, Sanofi, GSK, Thermofisher, Roche, Advanz, Infectopharm | No | Infectopharm, Pfizer | No | Member: Paul Ehrlich Society (PEG), board member, German Society for Infectious Diseases (DGI, advisory board), CAPNETZ (board member), German Respiratory Society (DGP, member), Robert Koch Institute (scientific advisor), German Society for Internal Medicine (DGIM, member), German Society for Pneumology (DSG, board member), scientific activity: pneumonia, sepsis, antibiotics, vaccinations, molecular pathogen epidemiology, clinical activity: hospital-wide infectious disease consultation service, antibiotic stewardship (ABS), participation in education/training: ABS courses (Medical Associations of Thuringia/Saxony), InfektioUpdate, DGI Summer School, course for physicians responsible for hygiene (Medical Association of Thuringia) | No COI |
| Quax, Tessa | No | No | No | No | No | No | Scientific activity: member of the German Association for General and Applied Microbiology (VAAM), Clinical activity: archaea, archaeal viruses, microbial viruses | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| Rajewski, Diana | No | No | No | No | No | No | Member: German Dermatological Society (DDG), the Professional Association of German Dermatologists (BVDD), the Rhenish-Westphalian Dermatological Society (e.V.), and the Lipoedema World Alliance | No COI |
| Rohde, Christine | No | No | No | No | No | No | Scientific activity: Principal investigator in the publicly funded projects Phage4Cure and PhagoFlow, no publications from either project; personal involvement in both projects dates back more than three years, with public funding for a postdoctoral position in each (at DSMZ, ended February 2019 and April 2020, respectively), no direct relevance to the guideline, co-supervision of the preclinical Clinical Leave stipend project IDEAL-EC at DSMZ (involved physician Dr. med. A. Y. Classen, University Hospital Cologne), period: April 2021 – September 2022, no publication to date, co-coordinator (together with Dr. Johannes Wittmann) of the preclinical, DZIF-supported project EVREA-Phage, no publication to date, currently member of three subgroups of the DZIF TransPhage Network and the Scientific Steering Committee, clinical activity: no clinical work, exclusively in vitro microbiology in the preclinical R&D sector, currently only involved in the DZIF-supported project EVREA-Phage (currently the only ongoing externally funded project) | No COI |
| Rupp, Markus | No | No | No | No | | No | Member: AO Trauma, German | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Society for Orthopaedics and Trauma Surgery (DGOU), European Bone and Joint Infection Society, Osteosynthesis and Trauma Care Foundation, scientific activity: bone regeneration, musculoskeletal infection, clinical activity: bone and joint infections, severely injured patients | |
| Ruth, Peter (Reviewer) | No | German Federal Chamber of Pharmacists | Presentations at conferences, symposia, and academic institutions | Scientific Publishing Company, Stuttgart | DFG | No | Member: German Society for Experimental and Clinical Pharmacology and Toxicology (DGPT), German Pharmaceutical Society DPhG, scientific activity: functional analysis of specific calcium and potassium channels as well as cGMP signaling in physiology and pathophysiology, clinical activity: none, participation in education/training: Phoenix Academy – continuing education for pharmacists, personal relationships: not applicable | No COI |
| Stahl, Mirjam | No | Vertex Pharmaceuticals | Vertex Pharmaceuticals | No | Vertex Pharmaceuticals; Boehringer Ingelheim | No | Member: FGM – Chairperson/Mucoviscidosis e.V., Treasurer GPP, Secretary Group CF, Assembly 7, ERS, scientific activity: cystic fibrosis, lung function diagnostics, lung imaging, clinical activity: cystic fibrosis | No COI |
| Turner, Paul | No | Felix Biotechnology | No | No | No | No | No | Topics relevant to phage research and therapy<br><br>COI moderate: Voting count with and without, Limitation of leadership function |
| Vehreschild, Maria J.G.T. | Maat, Tillotts Pharma, | No | Merck/MSD, 3M, Ferring, | No | HEEL, BioNTech, Roche, MSD | No | No | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | MSD/Merck, Roche, EUMEDICA, Ferring | | University Hospital Karlsruhe, University Hospital Cologne, Academy of Infectious Medicine, Klinikum Essen, Pfizer, University Hospital Heidelberg, University Hospital Frankfurt, State Medical Association of Hesse, Janssen, Institut Mérieux, Forum for Medical Continuing Education GmbH, University Hospital Freiburg, Berliner Dialy Seminar, ADKA, Falk Foundation, St. Johannes Hospital, DiaLog Service, CED Service, Medical Association of Lower Saxony, St. Josef | | | | | |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest, Classification by relevance, Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | Hospital, Limbach Group SE, SUMMIT OXFORD Ltd., EUMEDICA Kit Congress, Tillotts Pharma, Helios Hospitals, Lahn-Dill Clinics, GILEAD, GSK, Klinikum Leverkusen | | | | | |
| Weipert, Lisa | No | No | No | No | No | No | No | No COI |
| Willy, Christian | 3M, Mölnlycke | No | Mölnlycke | No | G-BA Innovation Fund | No | Member: German Society for Military Medicine and Military Pharmacy (DGWMP), German Society for Orthopaedics and Trauma Surgery, German Society for Surgery (DGCH), scientific activity: surgical site infection, prevention of infection, compartment syndrome, soft tissue injury, war wounds, antiseptics, clinical activity: surgical site infection, prevention of infection, compartment syndrome, soft tissue injury, war wounds, antiseptics, participation in education/training: wound treatment in war wounds, military medicine | No COI |
| Wittmann, Johannes | No | No | No | No | DZIF | No | No | No COI |
| Witzenrath, Martin | Aptarion, Pantherna, Treamid | No | Astra Zeneca, Insmed, Pfizer, Bayer | No | Biotest, Pantherna, Aptarion | No | Member: German, European, and American Society of Respiratory Medicine, German Critical Care Societies, German Society of Infectious Diseases (DGI), German Center for Lung Research (DZL), scientific activity: pneumonia, ARDS, | No COI |

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | clinical activity: respiratory medicine and critical care | |
| Würstle, Silvia | No | No | No | No | Third-party funding | No | Member: DGI, DGIM, DZIF, DGVS, ESCMID, BDI, Marburger Bund, Phage4-1Health, Bavarian State Medical Association, Association for General and Applied Microbiology, Phage Suisse, German Association of Women Academics, German Pharmaceutical Society, Circle of Friends of Water Chemistry and Chemical Balneology, Belgian Society Viruses of Microbes, scientific activity: infectious diseases, clinical activity: internal medicine, infectious diseases, participation in education/training: university lecturer | No COI |
| Ziehr, Holger | No | No | No | No | BMBF, GBA | No | Scientific activity: production of phages as biologics | No COI |
| Zumpfe, Sandra | No | No | No | No | No | No | Member: Federal Association of Organ Transplant Recipients (BDO e.V.), Chairperson | No COI |
| de Jode, Mathieu | No | No | No | No | No | No | Member: ISVM / International Society for Viruses of Microbes, scientific activity: PhD in microbiology with a focus on bacteriophage biology and phage therapy, currently working on quality control of clinical phage products used in Belgium, clinical activity: bacteriophage product safety | No COI |

1 As determined by the AWMF guideline, https://www.awmf.org/regelwerk/erklaerung-von-interessen-und-umgang-mit-interessenkonflikten:

- Coordinators of guideline projects should not have any thematically relevant conflicts of interest. In cases where this is unavoidable (e.g. because the expertise and commitment of the person concerned is indispensable), a co-coordinator without any thematically relevant conflicts of interest should be appointed (e.g. a methodologist or an expert in the field as a peer) or the guideline group be asked for deliberations and a decision.

- Participants with minor conflicts of interest should not hold any lead functions within the guideline development group (Members of steering committees/steering groups, working group leaders, persons mainly responsible for evidence processing, moderators). In cases where this is unavoidable, members without thematically relevant conflicts of interest should constitute the majority in steering committees, whilst it should be ensured that one member without thematically relevant conflicts of interest is appointed as a peer for individual functions.

| | Work as consult/expert | Work in a Scientific Advisory Board | Paid role as lecturer and/or educator | Paid authorship and/or co-authorship | Research projects/ conducting clinical trials) | Proprietary interests (patent, copyright law, share ownership) | Indirect interests | Guideline topics affected by conflicts of interest,<br>Classification by relevance,<br>Consequence |
|---|---|---|---|---|---|---|---|---|

Notes:

i. Prof. Li Deng was initially part of the guideline group. However, strong conflicts of interest were identified retrospectively, and - based on the recommendation of the AWMF team and in accordance with the AWMF regulations -she had to be withdrawn from the guideline group. She did not significantly influence the development of the guideline, and a recount of all recommendations and statements, excluding her vote, confirmed that the results remained unchanged.

ii. Dr. Mathieu de Jode participated in this guideline work solely during his affiliation with Sciensano, Brussels, Belgium.

# Supplementary File 2 - Template material transfer agreement (MTA)

This MTA is intended as a template to be adapted to the respective requirements and should not be used as is.

MTA Reference Number: _____

This Material Transfer Agreement ("MTA") is made with an effective date of [date, month, year] ("Effective Date")

**between**

**[PROVIDING INSTITUTION: Name, Address]**
-hereinafter called "**PROVIDER**"-

represented by [Name, Address]

Responsible Director of Department: [Name, Address]

**and**

**[RECIPIENT INSTITUTION: Name, Address]**

-hereinafter called "**RECIPIENT**"-

requested by RECIPIENT for use by its SCIENTIST

**[RECIPIENT SCIENTIST: Name, Address]**

- hereinafter called "**SCIENTIST"**-

**RECITALS**

A. Whereas, PROVIDER, a foundation under Public Law, possesses certain **MATERIAL** (as later defined herein) resulting from research conducted by the Investigator _____ as an employee of the PROVIDER.

B. Whereas, the RECIPIENT is interested in the use of the MATERIAL for the purpose of [Describe Purpose] by the SCIENTIST as described with the following details, hereinafter called the "**Purpose**":

   [Describe Purpose]

C. Whereas, the PROVIDER is willing to provide the RECIPIENT with the MATERIAL for the **Purpose** and use by its SCIENTIST under the terms and conditions set forth herein.

Now, therefore, the parties agree as follows:

**The MATERIAL**

The Material covered by this Agreement consists of:

[Describe MATERIAL]

hereinafter called "**MATERIAL**".

For the purpose of this Agreement, the term MATERIAL shall include the original MATERIAL, any Progeny, unmodified Derivatives, the original MATERIAL contained in Modification and proprietary information concerning the original Material.

*Progeny* shall mean any unmodified descendant from the MATERIAL (such as virus from virus, cell from cell, or organism from organism).

*Unmodified Derivatives* shall mean any substance created by the RECIPIENT that constitutes an unmodified functional subunit or product expressed by the MATERIAL (such as cloned/subcloned MATERIAL, purified or fractionated subsets of the MATERIAL, and proteins expressed from DNA/RNA, or monoclonal antibodies secreted by a hybridoma cell line).

*Modifications* shall mean any substances created by the RECIPIENT, which contain/incorporate the MATERIAL (such as crosses, breeding varieties, cell fusions, subcloning etc.).

The PROVIDER shall be free, in its sole discretion, to distribute the MATERIAL to other commercial and non-commercial entities and to use it for its own purposes.

1. **INVESTIGATIONAL LIMITATION**

The MATERIAL is made available to the RECIPIENT for the abovementioned Purpose only. The RECIPIENT agrees that the MATERIAL shall not be used for any other purpose, neither commercial nor other use. THE MATERIAL MUST NOT BE USED IN HUMANS IN ITS DELIVERED FORM.

2. **DISTRIBUTION AND RELEASE LIMITATION**

Neither the RECIPIENT nor the SCIENTIST shall distribute or release the MATERIAL to any other person or entity, except laboratory personnel under the SCIENTIST's immediate and direct control. The RECIPIENT shall ensure that no one will be allowed to take or send the MATERIAL to any other location than the SCIENTIST's laboratory, unless

- written permission is obtained from the PROVIDER, or
- the take-away or dispatch serves the purpose of [Describe Purpose]

3. **RIGHTS AND INTELLECTUAL PROPERTY**

The RECIPIENT agrees that furnishing the MATERIAL shall not grant any right, option or license under any patents, know-how or other intellectual property rights of the PROVIDER to use the MATERIAL for any products, processes or services for profit-making or other commercial purposes. The RECIPIENT ensures that the MATERIAL will not be used in research that is subject to consulting or licensing obligations to another institution, corporation or business entity.

4. **INVENTIONS AND PATENTS**

The RECIPIENT agrees that in the event the RECIPIENT makes any invention, improvement or modification, whether patentable or not, based on the MATERIAL as a result of activities conducted under the Purpose, the RECIPIENT shall promptly bring such invention, improvement or modification and any patent application filed thereon to the attention of the PROVIDER. The RECIPIENT shall grant the PROVIDER an irrevocable, non-exclusive, royalty-free license to practice such invention, improvement or modification for scientific research purposes. In case of joint inventions, the PROVIDER and the RECIPIENT shall conclude in good faith a separate agreement concerning the use, patenting and commercialization of such joint inventions.

5. **PUBLICATION**

The SCIENTIST and the RECIPIENT shall provide the PROVIDER, on a confidential basis, with a description of the results generated with the MATERIAL under the Purpose. If the SCIENTIST and the RECIPIENT desire to publish the results of the research, the SCIENTIST and the RECIPIENT will furnish the PROVIDER with a copy of the proposed manuscript or abstract thirty (30) days prior to publication. The PROVIDER may delay the publication if it contains patentable invention or confidential information. In both cases, the parties will discuss in good

faith how to proceed in order to protect the invention and/or confidential information. The SCIENTIST and the RECIPIENT shall acknowledge the source of the MATERIAL in any public or oral presentation.

6. **SECRECY**

The RECIPIENT shall keep all information related to the Purpose and/or the MATERIAL confidential irrespective of whether such information was received from the PROVIDER or generated by the RECIPIENT itself under the Agreement. Any oral disclosure from the PROVIDER to the RECIPIENT shall be identified as being confidential by written notice delivered to the RECIPIENT within thirty days after the date of the oral disclosure. This obligation is not applicable to information, which

- is publicly available at the time of the disclosure
- becomes publicly available after signature of this Agreement through no fault of the RECIPIENT
- is already in possession of the RECIPIENT without duty of confidentiality
- is required by law or regulation to be disclosed, or,
- is independently developed by the RECIPIENT without use of PROVIDER's information.

The RECIPIENT has to prove any of the aforementioned exceptions of confidentiality by appropriate written documentation.

7. **WARRANTY**

The MATERIAL is provided hereunder "**as is**" and is understood to be experimental in nature and may have hazardous properties. The MATERIAL shall be used with prudence and appropriate caution. The **PROVIDER MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND CONCERNING THE MATERIAL, EXPRESS OR IMPLIED, AND THE ABSENCE OF ANY LEGAL OR ACTUAL DEFECTS, WHETHER OR NOT DISCOVERABLE.** Specifically, and not to limit the foregoing, the PROVIDER makes no warranty or representation of merchantability or fitness for a particular purpose or that the use of the MATERIAL does not infringe any patents or other legal intellectual property rights of a third party, and that the use of the MATERIAL will not cause any damage of any kind to the RECIPIENT or a third party.

8. **LIABILITY**

In no event shall the PROVIDER be liable for any use by the RECIPIENT of the MATERIAL or any loss, claim, damage or liability, of whatsoever kind or nature, which may arise from or in connection with this Agreement or the use, handling or storage of the MATERIAL. The RECIPIENT will hold the PROVIDER and all of the PROVIDER's trustees, regents, officers, agents, and employees harmless and indemnify them for any loss or damage they may suffer from the RECIPIENT's (and his SCIENTIST's) use, handling, storage, or other activities connected with the MATERIAL.

If the RECIPIENT carries out [Describe Purpose] with the MATERIAL provided, the PROVIDER also bears no liability.

Should a legal liability case of the PROVIDER occur notwithstanding, the RECIPIENT hereby assures to indemnify the PROVIDER from any claims, including attorney costs incurred for the purpose of defense.

9. **REALISATION OF PROFITS**

The RECIPIENT of the MATERIAL undertakes to inform the PROVIDER immediately if the RECIPIENT realizes profits in the course of carrying out [Describe Purpose] with the transferred MATERIAL. In such a case, the Parties shall agree in a separate Agreement on profit sharing for the PROVIDER on fair terms.

10. **REGULATIONS/LAW**

The RECIPIENT and the SCIENTIST shall use the MATERIAL in compliance with all laws and guidelines and governmental regulations applicable to the MATERIAL.

This Agreement shall be construed and governed by the laws of the [COUNTRY]. The parties hereby submit to the exclusive jurisdiction of the courts of [COUNTRY]. Any dispute shall be brought before the competent courts of [Responsible court].

This MTA is not assignable. In the event the MATERIAL or part of it should be under physical control of the RECIPIENT or the SCIENTIST before the Agreement is signed, the terms and provisions of this Agreement shall apply for this MATERIAL retroactively.

11. **TERMINATION**

The RECIPIENT is allowed to use the MATERIAL during its research under the Purpose. However, the PROVIDER is entitled to request the immediate return or destruction of the MATERIAL, in case the RECIPIENT does not comply with its obligations under this Agreement or in any other case with two months prior notice.

The provisions concerning PUBLICATION, RIGHTS AND INTELLECTUAL PROPERTY as well as LIABILITY shall survive the expiration.

Authorized signatures of the RECIPIENT

……

Place, Date

……

[...]

RECIPIENT

*Certification of the RECIPIENT's SCIENTIST:*
*I have read and understood the conditions outlined in this Agreement and I agree to abide by them in the receipt and use of the MATERIAL.*

……

Place, Date

……

[...]

SCIENTIST

Authorized signatures of the PROVIDER

……

Place, Date

……

_____

President of _____

*Read and Acknowledged*:

……

Place, Date

……

_____

Investigator (if applicable)

……

Place, Date

……

_____

Responsible Director of Department

# Supplementary File 3 - Template informed consent form for patients

This consent form is intended as a template to be adapted to the respective requirements and should not be used as is.

## Information for Patients

Dear Patient,

Please read this document carefully. If you require further explanation or have any questions, do not hesitate to contact your attending physician.

Bacteriophage Therapy (Phage Therapy) for

_____

Date of Birth:

_____

## General Information on Bacteriophage Therapy (Phage Therapy) within the Framework of an Individual Treatment Attempt

**Bacteriophages (phages)** are viruses that specifically infect and destroy bacteria. They are considered a promising supplement to conventional antibiotic therapy, especially in the treatment of persistent bacterial infections. Their therapeutic potential is being intensively researched and already tested in initial applications in various countries, including Belgium, the USA, and Australia.

The medical use of phages dates back to the early 20th century. Since then, numerous case reports have provided evidence of successful treatments. However, as most phages act very specifically, systematic investigation through traditional clinical trials is only possible to a limited degree. Accordingly, phage therapy currently constitutes an unapproved, experimental form of therapy.

Phage therapy is offered to patients for whom previously administered standard therapies have not been sufficiently effective and who are acutely threatened by their illness or severely limited in their quality of life.

An individual treatment attempt is a form of therapy that deviates from the medical standard, with uncertain prospects regarding its success and safety. It is undertaken with the aim of curing diseases or at least alleviating their symptoms. The acquisition of scientific knowledge is secondary. Such treatment attempts are only undertaken when, based on the physician's assessment, scientific understanding, and medical experience, there is a reasonable prospect of treatment success.

To determine whether you are eligible for experimental phage therapy, a risk-benefit assessment will be carried out based on detailed medical history and diagnostic findings. This includes information on your medical history, laboratory and vital parameters, and microbial samples. The method, frequency, and duration of administration will be determined by your

attending physician. Provided your informed consent or that of your legal representative, phage therapy will be administered in the hope of restoring your health or alleviating your symptoms.

## Potential Benefits

Due to the antimicrobial effect of phages, a positive impact on your condition may occur. This experimental therapy could improve your symptoms and quality of life and, in some cases, may even lead to a cure. Through the targeted selection of phages, antibiotic therapy can also be supported, potentially restoring its effectiveness. However, as this is an experimental therapy, the potential benefits for you cannot be clearly predicted. The treatment may not result in any improvement.

The planned treatment is intended solely to restore or improve your health. No measures are undertaken or omitted solely for the purpose of gaining scientific knowledge.

## Possible Risks

Please note that phage therapy is an experimental and not yet standardized form of treatment. Although initial clinical studies and numerous case reports suggest good tolerability, not all potential risks and side effects are fully known at this time.

**Acute Reactions During or After Application:**

- During treatment, increased body temperature up to **fever, chills, malaise, skin redness, headaches, or limb pain** may occur. These symptoms can be associated with the intended killing of bacteria and the resulting release of bacterial components.
- There is a risk of **allergic reactions or intolerance** to components of the phage preparations. These may manifest as skin rashes, shortness of breath, nausea, or, in rare cases, anaphylactic shock. Intensive medical care may be required in such cases.
- In very rare instances, **serious complications** such as **acute organ failure, neurological damage** (e.g., seizures, altered consciousness), or **even death** may occur. Symptoms will be closely monitored throughout the therapy.

**Risks Related to the Effect of Phages:**

- When a large number of bacteria are destroyed at once, your body may **respond strongly to the bacterial components** released during this process.
- Target bacteria may **develop resistance** to the applied phages, which could reduce the effectiveness of the treatment.
- In some cases, a change in phage strains (sequential therapy) may be necessary if resistance occurs. However, this requires that suitable **alternative phages are available** – which cannot always be guaranteed.
- In rare instances, the **pathogenicity (disease-causing potential)** of bacteria may unfavorably change, for example, through altered antibiotic susceptibility. This could theoretically lead to an infection that is more difficult to treat.

- Repeated or prolonged administration may lead to an **immune response against the phages** themselves. This could impair the therapy's effectiveness. The long-term effects of such an immune response are currently not fully understood.

**Other Potential Risks:**

- The **effectiveness** of phages can vary depending on manufacturing, storage conditions, and the individual bacterial profile. Activity differences between product batches are possible.
- Despite careful production processes, traces of contaminants may remain, such as bacterial cell debris, endotoxins (toxins from bacteria), plasmid residues, or allergenic components. These may cause adverse reactions. This particularly applies to **residual bacterial components** from phage production (e.g., bacterial cell wall components, endotoxins).
- In rare cases, phages may **transfer genetic material** between bacteria. This could theoretically result in the transmission of harmful traits (e.g., toxins or antibiotic resistance genes) to other bacteria.
- Potential **interactions with other medications** cannot be ruled out.
- **Delay** of other effective treatments: The decision to proceed with phage therapy may in some cases delay or preclude the timely application of other proven therapies (e.g., certain antibiotics or surgical procedures).
- Application to specific areas of the body (e.g., central nervous system, intra-articular, intravenous) carries **organ- or site-specific risks**, which will be explained separately on a case-by-case basis. Depending on the **method of administration** (e.g., intravenous, local, inhalational, intra-articular), specific risks may include infections, irritation, pain, tissue damage, or allergic reactions at the application site.
- Phage treatment may also affect beneficial bacteria on the skin, mucous membranes, or in the gut (**microbiome**). This could disturb the natural balance and promote infections or digestive issues. However, the expected impact on the microbiome is generally lower than with antibiotic therapy.
- The following substances/excipients not approved for human use are contained in the preparation (e.g., to improve solubility):

  _____

  These may pose potential risks and are therefore disclosed here.

**Unknown Long-Term Effects:**

As this is an experimental therapy, long-term effects on the immune system, microbiota, and overall health have **not yet been fully investigated**. The risk of currently unknown side effects cannot be excluded.

### Additional Risks Specific to Your Case:

_____

_____

### Therapy Modifications During the Course of Experimental Treatment

Based on the progression of your infection or your clinical condition, your treating physician may find it necessary to adjust the treatment. This may include changes to the dosage, duration, method, or frequency of administration. Temporary or permanent discontinuation of the therapy may also become necessary.

Your physician may also decide to modify the composition of the phages and/or alter any combination with antibiotics. You will be informed of any changes made to your treatment.

**You have the right to discontinue the therapy at any time, without providing any reason.**

### Follow-up Care

Follow-up care after the experimental therapy corresponds to that of standard treatment for bacterial infections, but with an increased frequency of monitoring. This includes regular clinical, laboratory, and microbiological evaluations.
For your safety, we recommend that you inform us of any changes in your health status within the first 12 months after the end of treatment.

### Possible Treatment Alternatives

Alternatives or complementary therapies to experimental phage therapy may include:

- Treatment with antibiotics and other antimicrobial agents
- Surgical interventions
- Additional treatment alternatives specific to your case:

_____

The feasibility of the above-mentioned alternatives and/or complementary treatments depends on the type and characteristics of the specific infectious disease. We are available to advise you on these options.

### Costs Associated with Phage Therapy

*[Explanation of how cost coverage or reimbursement is handled in your specific case.]*

### Data Protection and Privacy

Information about your illness and treatment will be shared with our internal and external staff and contractual partners, for example as part of the Phage Therapy Board during case

discussions. Clinical and microbiological samples will also be collected, documented, and stored throughout the treatment period. This is primarily for your safety and to better understand the course of your treatment.

Documentation will follow scientific standards and may be published and/or registered in national and/or international databases in anonymized or pseudonymized form. All patient-related data will be encrypted, and data protection regulations will be strictly observed.

*[Insert applicable data protection regulations and contact details of the Data Protection Officer.]*

## Your Contact Person

Your contact person for questions or in case of unexpected reactions during or after the treatment is:

_____

## Informed Consent Form

Bacteriophage Therapy (Phage Therapy) for _____

Date of Birth: _____

**By signing this document, I confirm that:**

- My treating physician _____ has informed me both orally and in writing about the experimental treatment with phage therapy and the course of therapy. I have received and read the patient information and consent form related to the above-mentioned project.
- I have been informed about the possible benefits and risks of the treatment. I have also been made aware of alternative therapy options.
- I was given the opportunity to ask questions, which were answered fully and understandably.
- I agree that my medical data and microbial samples may be shared with internal and external staff as well as involved contractual partners.
- I have been informed that the scientific evaluation of the therapy and its treatment outcomes is secondary to my medical care and that my well-being is always the primary concern. I expressly declare that I consent to a scientific analysis of the therapy and the resulting treatment outcomes.
- I agree that the data collected during the experimental therapy, especially health data, may be used for scientific purposes, such as anonymized or pseudonymized publication as a case report. Furthermore, I consent to the storage of information gathered during my therapy in a scientific database.
- After sufficient consideration, I agree to receive the experimental therapy under the stated conditions. My consent is given voluntarily.
- I understand that I can revoke my consent at any time without providing reasons.
- I have been given a copy of the written information and consent form, or I will receive one after signing.

**Patient (Last Name, First Name)**:

_____

**Place, Date**:

_____

**Signature Patient**:

_____

**Physician (Last Name, First Name)**:

_____

**Place, Date**:

_____

**Signature Physician**:

_____