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Review

Phage Therapy Annotated Glossary

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Abstract

Bacteriophages, or phages, are the viruses of bacteria. Bacterial viruses have been used as antibacterial agents, including clinically, approximately since their discovery, now over 100 years ago. In this age of increasing antibiotic resistance, along with concerns over the health impacts of unintentional microbiome modification due to the use of relatively broad spectrum antibiotics, the idea of using comparatively narrow-spectrum, diverse, and abundant bacteriophages as antibacterial agents has come back into fashion. In fact, the use of phages clinically as antibacterial agents never completely went away, and phages otherwise have been used as antibacterial agents over the decades by apparently millions, particularly in the former Soviet Union. In the course of these efforts, a certain terminology has developed in association with phage therapy, or as has been coopted from more general phage biology to the use of phages as antibacterial agents. Many of these terms and associated concepts, however, are relatively obscure or, in many cases, seemingly misunderstood. Consequently, here I provide a list of phage-therapy relevant terms and definitions, along with associated discussions of phage therapy from the perspective of its terminology, all as written from a phage-therapy pharmacological perspective. The hope is to achieve a more efficient and effective development of phage therapy technologies through a more consistently comprehensible application of concepts and terminology.

Key Words

Antibacterial, Bacteriophage Therapy, Biocontrol, Biological control, Phage Therapy, Pharmacology

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Introduction

The official discovery of bacteriophages as antibacterial agents occurred at a time, the mid 1910s [1-4], when selectively toxic antibacterial therapeutics were extremely limited, this being over a decade prior to the discovery of penicillin in the late 1920s [5], and well prior as well to the first clinical implementation of antibiotic therapy [6]. This was also nearly three decades before widespread antibiotic use, starting in 1945 [7,8]. Even so, the early years of clinical phage therapy [9-13] does not appear to have been implemented to a degree that has been in any way as widespread as antibiotics have come to be used. Indeed, the eventually extensive use of antibiotics in the 1940s seems to have contributed to declines in enthusiasm for phage therapy [9]. Phage therapy, however, was not completely lost from clinical practice, but instead has persisted in everyday use especially in the former Soviet Union [14,15].

Today, though still quite limited in its clinical practice outside of the former Soviet Union, there has been a resurgence in enthusiasm for phage therapy [16-23]. This has been seen particularly as the usefulness of antibiotics has increasingly waned, due especially to the evolution of antibiotic resistance by bacterial pathogens [24], but also due to increasing awareness of the importance of our microbiomes [25] along with their fragility in the face of broad-spectrum antibiotic use [26]. Successful redevelopment and deployment of phage therapy, however, requires a robust appreciation of the biology of phages and, indeed, of the pharmacology of phage therapy [27-33].

Toward these ends, it would be helpful for researchers as well as practitioners to speak a common, mutually understood technical language. Here I address especially the issue of phage therapy-related terminology, and particularly that of the terminology of phage therapy pharmacology. The goal is not only to provide facile access to definitions but also to discuss common misconceptions as have come to my attention [34-37]. See also Adriaenssens and Brister [38] and Aziz *et al.* [39] for discussion of issues concerning phage naming and phage bioinformatic analysis respectively. For access to the phage therapy literature more generally, see Alves and Abedon [40,41]. In particular, I provide here a phage therapy glossary with a pharmacological emphasis and extensive annotation.

Annotated Glossary

Here I present a glossary of phage therapy-relevant terms, with focus explicitly (i) *not* on those terms which are pertinent only to the study of phage biology more generally, (ii) *not* on Enzybiotics [42], and also (iii) *not* on more general issues of drug development, but instead with focus especially on pharmacological aspects of whole-phage use as antibacterial agents. Definitions and associated discussions are provided in term-alphabetical order, and the glossary is annotated for the sake of increasing perspectives as well as addressing common misconceptions. Unless otherwise indicated, the term “Phage therapy” is used to imply clinical as well as more environmental, that is biological-control use of phages as antibacterial agents [43]. In addition, the terms “therapy” and “treatment” mostly are used interchangeably. Note that pharmacokinetics refers to the impact of bodies on drugs, particularly as affecting drug densities within specific locations within bodies, and includes processes known as Absorption, Distribution, Excretion, and Metabolism [27,28,30], all as briefly considered here from a phage therapy perspective.

Additional glossaries of phage and phage-related terms can be found in Benzer *et al.* [44], Lwoff [45], Tolmach [46], Adams [47], Hershey [48] – the latter as generated by Ira Herskowitz, [49] – Rieger *et al.* [50], Birge [51], Kutter [52], Abedon [53-56], Abedon *et al.* [57], Hyman and Abedon [58], and Dąbrowska *et al.* [59]. The latter eight publications can be viewed as precursors to the glossary presented here. See also the ACLAME Phage Ontolology [60] along with a number of general reviews of phage therapy pharmacology [27,59,61]. For a listing and discussion of ‘poorly used’ phage terms, see Abedon [62].

An assumption is made that the glossary will be read primarily piecemeal rather than necessarily in the presented order from start to finish. Toward reducing redundancy in defining subsidiary terms within definitions and discussions, those terms that are found elsewhere in the glossary have been capitalized as a navigation aid.

Nevertheless, for the sake of readability, I have not completely eliminated such redundancy. The following thus is a phage therapy annotated glossary, with an explicit aim of increasing the collective appreciation of the meanings of phage therapy-relevant terms and concepts.

As the hope is to treat this glossary as a 'living' document prior to its eventual formal publication, please contact me with any thoughts that you might have on how the glossary might be improved. This includes perhaps especially references that you feel should be included as much of the referencing made here was done when the manuscript was initially drafted, in 2018. Please include where explicitly it should be placed and any wording that should be placed around it. In other words, help me out as much as you possibly can when suggesting references to add!

Abortive Infection

Abortive Infections by phages are associated with both bacterial death (Bactericidal Infection) and low phage Efficiency of Plating (EOP). Generally this means that either no or few Virion Particles are produced per aborted phage infection of a bacterium. Abortive Infections can be a consequence of phage defects (i.e., phage mutations or instead phage nucleic acid damage), genetic incompatibilities between a wild-type infecting phage and an Adsorbed bacterium, otherwise poor bacterial physiological states (e.g., stationary phase), bacterial defense strategies (i.e., abortive infection systems), or simply infection circumstances. The latter may include high-phage-multiplicity infections that, in some manner, come to overwhelm the capacity of an Adsorbed bacterium to support a phage Productive Infection.

Review of Abortive Infection Systems as well as overviews of other mechanisms of bacterial resistance to phages can be found elsewhere [63-66] and phage mechanisms of resistance to bacterial defense strategies have been reviewed as well [66-68]. For a perspective of Abortive Infections particularly from a bacterial evolutionary ecological perspective, see [69]. Two related but not identical phenomena, discussed as follows, are phage inactivation by restriction endonucleases and the phenomenon of Lysis from Without.

Not Action of Bacterial Restriction Endonucleases

Contrast the concept of Abortive Infections with the consequence of restriction endonucleases action on infecting phages. Such phage restriction has the effect of blocking phage infection, but unlike with Abortive Infections the infected bacterium survives [70]. Abortive Infections can be sufficient to allow for successful Phage Therapy, since phage-Adsorbed bacteria by definition are killed even if they don't necessarily

support the production of additional Phage Particles (contrast, that is, Passive Treatment with Active Treatment). Infections where phages are restricted while Target Bacteria are not killed, however, cannot give rise to successful Phage Therapy.

Usually not Lysis from Without

The process of Lysis from Without resembles an Abortive Infection since both Adsorbing phages and Adsorbed bacteria do not survive the process. It is important to recognize, however, that not all Abortive Infections, even if associated with high phage Multiplicities of Adsorption, are necessarily a consequence of Lysis from Without. Indeed, phage Bactericidal Infections which are also not phage Productive Infections should be assumed by default to represent Abortive Infections rather than necessarily representing products of Lysis from Without—at least absent additional evidence supporting this latter interpretation, such as observation of very early phage-induced bacterial Lysis. Nevertheless, it is fairly common in the literature for Lysis from Without rather than Abortive Infection to be invoked, without evidence, given observations of bacterial death in association with high phage Titers. Note that Lysis from Without is discussed further below as its own glossary entry.

Absorption (pharmacokinetics)

Absorption in terms of pharmacokinetics is movement of medicaments into the blood. This is associated with systemic delivery to the body. For Phage Therapy, this can be accomplished directly, i.e., intravenously [71], less directly via phage application first to a within-body compartment (e.g., intraperitoneally or intramuscularly), or instead through phage delivery to the post-stomach GI tract, lungs, or even rectum. See as well Bacteriophage Translocation. Routes of Phage Therapy delivery more generally are discussed by Ryan *et al.* [72], and see also [28-30].

Active Infection

An Active Infection, from the perspective of Phage Therapy, is either a Productive Infection, by a phage of a bacterium, or at least a bacteriolytic or Abortive phage infection. Contrast Active Infections therefore with phage infections which, especially, do not result in bacterial death, i.e., particularly restricted infections in which the infecting phage does not survived but the infected bacterium does [70]. As a matter of degree, contrast also with infections which give rise to Lysogenic Cycles. The concept of Active Infection is relevant toward appreciating use of the term ‘active’ in the concepts of Active Treatment or Active Penetration, though in this case it is particularly Productive Infections that are involved.

Active Penetration

Active Penetration refers to the idea that phages can serve as effective anti-biofilm agents particularly due to the phage ability to Actively Infect Target Bacteria. The result, minimally (and ideally), is Lysis of those bacteria which have become phage infected. In addition, and probably useful as well to phage anti-biofilm efficacy, phages also typically can generate new phages in the course of such Active Infection (resulting, i.e., in Productive Infection), thus giving rise to Auto Dosing, that is, *In Situ* phage generation of new Phage Particles. So-produced phages may then penetrate to bacteria that are adjacent to Productively Infected bacteria, as found within the same biofilms [73]. The latter can be described also as a treatment which is active on more local versus more global distance scales (see Active Treatment—Locally Active Treatment).

Note that biofilms, and perhaps particularly more mature biofilms, may possess mechanisms of resistance to this Active Penetration [74,75]. Biofilms also can possess mechanisms of resistance simply to virion Penetration into biofilms, e.g., Vidakovic *et al.* [76]. For access to the phage-treatment-of-biofilms literature, as well as overviews of the possible ecology of those interactions, see Abedon [11,77] and Abedon *et al.* [78].

Active Treatment (Active Therapy)

Active Treatment, or Active Therapy, is an approach to Phage Therapy that is dependent on Auto Dosing, that is, on *In Situ* phage generation of new Phage Particles, and particularly as resulting *In Situ* phage Population Growth. With Active Treatment, fewer phages are applied than would be required to Adsorb most Target Bacteria. These phage numbers are then amplified in association with phage-infected Target Bacteria via Productive Infections to densities that are sufficient to result in infection of most of these bacteria, that is, ideally increasing in numbers to phage Inundative Densities or, at least, to what can be described as phage Clearance Thresholds.

Contrast the concept of Active Treatment especially with Passive Treatment. To a lesser degree, contrast Active Treatment also with Active Penetration. Note furthermore that successful Active Treatment may be equated with what is known phage ecologically as “Kill the winner” [79-81]. That is, Active Treatment requires Target Bacteria to be present at sufficiently high concentrations – that is at “Winner” densities – to support phage Population Growth to densities that are capable of inundating and thereby killing bacteria (i.e., minimally to above Clearance Thresholds and ideally to Inundative Densities). Alternatively, see the concept of Numerical Refuge, which would represent the presence of Target Bacteria at densities which by definition are not able to support successful Active Treatment.

Sufficient phage numbers to result in substantial bacterial eradication should be assumed to be somewhat in excess of existing numbers of Target Bacteria, e.g., a minimum of about ten phages for every one Target Bacterium, and this is rather than simply one phage for every bacterium. In addition, these phages must adsorb bacteria rather than simply be found in the presence of Target Bacteria (and thus not simply as specified by MOI_{input}). See Multiplicity of Infection, Multiplicity of Adsorption, and Poisson Distribution for further discussion of these latter points. For further discussion of Active Treatment, see Payne *et al.* [82], Payne and Jansen [83,84], and also Abedon and Thomas-Abedon [27]. See also the concept of Mixed Passive/Active Treatment. In addition, consider below the relatively novel concepts of Globally Active Treatment versus Locally Active Treatment, along with issues associated with inferring the occurrence of Active Treatment. For an Active Treatment online calculator, see [85].

Active Treatment—Globally Active Treatment

Globally Active Treatment [32,86] is Active Treatment as normally defined (above), i.e., as considering especially its occurrence in well-mixed broth cultures. Within a given compartment, or across an entire treated environment, phages thus must come to reach Inundative Densities via Population Growth or at least exceed Clearance Thresholds to result in somewhat successful Active Treatment. Globally Active Treatment likely is an ideal rather than a description of Phage Therapy as it typically occurs, however. That is, in quantitative terms Globally Active Treatment is more a theoretical construct and/or something that tends to occur over only relatively small volumes, unless larger volumes are well mixed, e.g., as might be seen within circulating blood.

Active Treatment—Locally Active Treatment

Locally Active Treatment [32,86] refers to the potential of a phage population to reach Inundative Densities, or at least exceed Clearance Thresholds, over much smaller spatial scales than an entire environment. This potential for phages to locally reach Inundative Densities would occur as a consequence of low amounts of environmental mixing, which can allow phage densities to build up locally in association with nearby high densities of bacteria. *Local* here especially refers to over sub-millimeter spatial scales, e.g., such as over a single bacterial microcolony or over a relatively small portion of a bacterial biofilm. To the extent that the latter involves a linkage between ongoing Auto Dosing, i.e., *In Situ* phage population growth, and local phage Penetration into a bacterial biofilm or microcolony, then Locally Active Treatment and Active Penetration describe equivalent phenomena.

Inferring Active Treatment

A variety of measures may be used to infer the occurrence of *successful* Active Treatment (rather than successful Passive Treatment), some preferable to others. The key indicators are application of insufficient phage numbers to achieve substantial Adsorption to Target Bacteria by the supplied phages (i.e., not in excess of the phage Clearance Threshold), this in combination with evidence of both *In Situ* phage Population Growth and subsequent substantial bacterial eradication. Merely the formation of new phage virions *In Situ* is not sufficient to imply successful Active Treatment, however. Nor is demonstration even of phage Population Growth *In Situ*, or of some bacteria killing, as none of these indicators explicitly show that *sufficient* phage Population Growth had occurred to achieve *substantial* bacterial eradication, that is, for phages to have exceeded their Clearance Threshold or reached Inundative Densities despite not having been originally dosed with those phage Titers. Formation of new virions nevertheless is an indicator of phage Productive Infection, which in turn serves as a requirement for Active Treatment. Inferring Active Treatment thus requires demonstration of *In Situ* increases in phage Titers to at least phage Clearance Thresholds and ideally to phage Inundative Densities. For further discussion along these same lines, see Killing Titers—Application of Concept.

What does 'Active' Mean in this Context?

I have suggested elsewhere [27,87] that the term 'active' within the context of Active Treatment is probably referring to the activity of the phages, i.e., with phages required to *Actively* Infect bacteria to achieve Active Treatment, whereas with Passive Treatment – which by definition does not require *In Situ* phage Population Growth – no such active infection is required. To a large extent this is confusing because with Passive Treatment the treating individual, such as a physician, is in fact more 'active' in that treatment, that is, responsible for achieving all of a resulting phage *In Situ* Titer, whereas with Active Treatment the treating individual (e.g., a clinician) is actually less actively involved in establishing that *In Situ* phage Titer.

An alternative interpretation, and one that I have come to favor, is that the contrast between Active Treatment and Passive Treatment stems instead from terminology used in immunology. There, active refers to the presence of effector cells, especially antibody-producing cells, whereas passive refers to a lack of such cells. Thus, active immunity occurs following exposure to a pathogen or instead to a vaccine such that lymphocyte memory cells are formed. With passive immunity, by contrast, only antibodies are transferred, e.g., as seen in association with serum therapy or via the ingestion by newborns of colostrum.

In this immunological contest, Active Treatment also involves cells. That is, phage-infected bacteria produce new phages *In Situ*, just as plasma cells produce new antibodies *In Situ*. Similarly, Passive Treatment does not involve cells in this same context. Instead, all of the phages that will ever need to exist for treatment to be successful will, given at least Purely Passive Treatment, have been supplied in the course of dosing and thus will not involve cells *In Situ* in terms of at least a requirement for new phage production. Equivalently, with passive immunity, all of the antibodies that will ever be present are, at least in principle, being supplied via dosing, with no subsequent antibody production *In Situ*, or at least antibody production associated with that antibody treatment, or instead with colostrum consumption.

Adsorption

Adsorption [88-91] is the process of phage virion acquisition of host bacteria. Steps involved in phage Adsorption include an ordered combination of extracellular virion diffusion (that is, an extracellular 'search' for bacteria to infect), Encounter of a virion with the surface of a bacterium, various generally somewhat specific interactions between virion proteins and bacterial surface molecules, and changes in virion conformation which result ultimately in virion Attachment to the surface of a bacterium. The latter is then followed by virion nucleic acid translocation into the bacterial cytoplasm, though this latter step is not necessarily included when referring strictly to virion Adsorption.

Adsorption, importantly, is not identical to simply phage addition to environments (see, e.g., Multiplicity of Adsorption). In addition, Free Phages do not necessarily end up becoming Attached to bacteria even given Encounter with bacteria (see Adsorption Affinity as well as Host Range). Furthermore, a time lag will exist between phage application (dosing) and phage Attachment. Adsorption, post-Encounter with a bacterium, also may be distinguished into reversible and irreversible aspects, with reversible adsorption preceding irreversible adsorption in the virion Attachment process [92]. Nucleic acid translocation, as well as molecular aspects of infection processes more generally, typically can be viewed as 'black boxes' from a phage therapy perspective, so consequently are not addressed in detail here. For more on adsorption, see [88-91,93-95]. See also an online phage Adsorption calculator at [96].

Contrasting Attachment, Adsorption, and Infection

Adsorption appears to be used by many authors equivalently to simply the Attachment of virions to bacteria. Thus a phage can be said to have Adsorbed a bacterium (meaning Attached) whereas the Adsorption *process* involves both virion

diffusion and various post-bacterial Encounter but pre-irreversible Attachment steps. Adsorption, that is, can but will not always be viewed as a broader concept than that of the Adsorption end point of Attachment.

The term 'infection' also is often used in a manner which is not greatly differentiated from that of Adsorption. For many authors, consequently, 'adsorption' by a virion will be described instead as 'infection' by a virion, even if nucleic acid translocation has not necessarily occurred, and indeed even if it is the process of virion Attachment which his being emphasized. This tendency presumably stems historically from a time before it was understood that not all phage Adsorptions necessarily resulted in phage infections, such as prior to appreciation of the concept of superinfection exclusion [97-99]. Compare thus the concepts of Multiplicity of Infection and the arguably more correctly stated but little used concept of Multiplicity of Adsorption, as well as differences between Secondary Infection (as considered here in a 'Biomedical Sense') and secondary *adsorption*. Even among Adsorbed phages which do succeed in infecting, not all of those infections will be Productive – e.g., see Abortive Infection – nor even necessarily Bactericidal.

Adsorption Affinity

Following virion Encounter with a bacterial surface, Adsorption Affinity is measured in terms of the likelihood, that is, the probability that subsequent virion Attachment will occur. As such, Adsorption Affinity contributes to the magnitude of Adsorption Rate Constants [100], with higher Adsorption Affinity resulting in greater Adsorption Rates. Generally, it is considered to be desirable for phages during Phage Therapy to display greater Adsorption Affinities for Target Bacteria rather than lower affinities, as thereby every phage-to-Target Bacterium Encounter has a higher probability of resulting in phage Adsorption and thus subsequent Bactericidal Infection. Note, though, that as Adsorption Affinity is a post-Encounter aspect of phage Adsorption. It therefore should be mostly independent of the target size of individual bacteria, as bacterium size affects virion-Encounter likelihood and this is rather than affecting virion Attachment likelihood following Encounter with a bacterium.

Adsorption Affinities of specific phage types can vary as a function of Target Bacterium properties, i.e., bacterial genetics as well as physiology. Variation can even in principle occur across a single bacterial population, thereby giving rise to 'physiological refuges' or 'phenotypic resistance' for a fraction of bacteria [101-103]. Adsorption Affinity can also vary as a function of environmental factors as can affect not only bacterium properties but virion properties as well—for the latter, see Adsorption

Cofactor. Additional discussion of Adsorption Affinity from an phage-ecological perspective is presented by Chan and Abedon [104].

Adsorption Cofactor

An Adsorption Cofactor is a small molecule or ion that contributes to virion Adsorption Affinity. Adsorption Cofactors typically will include divalent cations (such as Ca^{2+} and Mg^{2+}) or monovalent cations (such as Na^{+} or K^{+}), but also can include organic factors such as tryptophan [89,105]. In addition, temperature, pH, and osmolarity can impact virion adsorption characteristics [106]. Differences in phage Adsorption Rates and therefore in Adsorption Rate Constants thus can exist between environments as a function of the chemical and physical properties of those environments. As a consequence, there is a potential utility for making efforts to duplicate *In Situ* conditions for *In Vitro* phage testing. That is, it is not always certain that Adsorption Rates as measured *In Vitro* using standard laboratory media and conditions will be equivalent to Adsorption Rates as could occur *In Situ*.

Adsorption Rate

There are two relevant perspectives on phage Adsorption Rates, differing in terms of what is being emphasized as Adsorbing, the phage or instead the bacterium. These are either (1) the duration of Phage Particle transition from a Free Phage state to an irreversibly Adsorbed state or, alternatively, (2) the rate of transition of bacteria from an unadsorbed state to a phage virion-Adsorbed state. In general for Phage Therapy it is the latter rather than former perspective which is most relevant, i.e., time spent as a Free Phage. It is generally preferable for Phage Therapy also to achieve higher rather than lower Adsorption Rates.

Increasing Adsorption Rates

Adsorption Rates are a function of a combination of virion diffusion rates, virion Adsorption Affinity for the Target Bacterium, and bacterial target size: collectively, these define a phage's Adsorption Rate Constant. Adsorption Rates thus can be increased *In Situ* especially by selecting for faster-Adsorbing phage variants, i.e., as Bred Phages displaying greater Adsorption Affinities. This will tend to have more utility, however, only if starting with somewhat low Adsorption Rate Constants, and beware also that increasing a phage's Adsorption Rate for one bacterial strain may have negative consequences on that phage's Adsorption Rate for other bacterial strains, an example of a more general concept known as antagonistic pleiotropy [107-110]. It is possible also to compensate for lower Adsorption Rates to Target Bacteria, in terms of rates of bacteria

transition from unadsorbed to Adsorbed states, simply by supplying more phages (higher Titers), just as catalyzed reactions can be increased in rates simply by supplying more catalyst [43].

Adsorption Rates can be enhanced, as noted, by increasing densities of Free Phages, or instead by increasing densities of Target Bacteria, but those approaches are not equivalent. Higher Adsorption Rates for individual phages in particular are seen (1) given higher densities of adsorbable bacteria within an environment along with Adsorption Rate Constants of greater magnitude. Alternatively, (2) the rate at which an individual bacterium will become Adsorbed is a function of Free Phage densities, i.e., of their Titer, again in combination also with the magnitude of the phage's Adsorption Rate Constant. This is rather than as a direct function of densities of Target Bacteria. As it is the latter, adsorption of bacteria by phages, which is the primary goal of Phage Therapy, achieving higher Adsorption Rates for phage-based treatments consequently is not usefully accomplished by allowing Target Bacteria to increase in numbers. That is, increasing bacterial densities has the effect of increasing rates that phages adsorb to bacteria (measured as rates of loss of Free Phages) rather than rates at which bacteria are Adsorbed by phages (measured as rates of loss of phage-uninfected bacteria). It especially is the rate of transition of bacteria from unadsorbed to Adsorbed states which is relevant to Phage Therapy success, however.

Note that Target Bacteria exceeding Proliferation Thresholds nevertheless still is relevant to Active Treatment success, thus implying a utility to higher versus lower bacterial densities for Phage Therapy success, at least under certain circumstances. The relevance of Target Bacteria reaching such densities is less a function of phage Adsorption Rates, however. Instead, this is a function especially of the potential of these bacteria to support phage Population Growth to Inundative Densities in the course of Auto Dosing. Particularly, peak *In Situ* phage Titers as a consequence of phage Population Growth will tend to be determined as a product of Target Bacterial densities and a phage's Burst Size, rather than as a function strictly of rates of Free Phage Adsorption to Target Bacteria.

Adsorption Rate Constant

An Adsorption Rate Constant is a measure of the per capita likelihood of Free Phage Attachment to a given Target Bacterium. This measure can be viewed as the probability that Attachment will occur given the suspension of a single virion along with a single Target Bacterium within a specific volume, as occurring over a given length of time. Contrast with simply Adsorption Affinity, which is the probability of virion Attachment given virion Encounter, that is, as follows Phage Particle collision with a

bacterium. Adsorption Affinity, however, is a component of Adsorption Rate Constants. Contrast also simply Adsorption Rate, which is the product of the phage Adsorption Rate Constant and the density of Adsorption targets, as considered further below.

Adsorption Rate Constant units can be one ml and one min or, as many prefer, one ml and one hour. If you multiply this probability by the density of bacteria present, then you will obtain an estimate of the probability that a given virion will adsorb over that time frame while in association with a given density of Target Bacteria. Alternatively, multiply the Adsorption Rate Constant by the density of phages present and you will be estimating the per bacterium probability of becoming phage Adsorbed, in each case over the unit time frame, i.e., 1 min or 1 hour. For description of how to calculate Adsorption Rate Constants, see Hyman and Abedon [111].

Using Adsorption Rate Constants

For an Adsorption Rate Constant of 2.5×10^{-9} ml^{-1} min^{-1} [100] and 10^6 phages/ml, then an approximation of the likelihood that a given bacterium will become phage Adsorbed over 40 min is $2.5 \times 10^{-9} \times 10^6 \times 40 = 0.1$, that is, Adsorption Rate Constant multiplied by phage Titer multiplied by time. More precisely, this probability is equal to $1 - e^{-2.5 \times 10\text{^}-9 \times 10\text{^}6 \times 40}$, where the exponent is equal to MOI_{actual}, which takes into account that not every virion Adsorption over a given span of time will be to a bacterium which has not yet been phage Adsorbed. For further clarification of the latter calculation, see Poisson Distribution as well as Multiplicity of Infection. It is also possible to calculate a phage half-life in association with a given density of target bacteria for a specific Adsorption Rate Constant [112]. See also Bacterial Half Life.

To perform these calculations, it is crucial to accurately determine Adsorption Rate Constants for a given phage, bacterial strain, and conditions. Note, however, that Adsorption Rate Constants cannot be determined accurately using only end-point Adsorption Rate-determination experiments, which involve comparing only a given starting Free Phage concentration with a given ending Free Phage Concentration [92], and this issue is particularly relevant if Free Phages are separated from phage-Adsorbed bacteria via artificial Lysis of the latter or if phage-induced Lysis from within can possibly occur within the time-frame of an experiment. That is, multiple time points – ideally indicating exponential changes in numbers of unadsorbed (Free) phages over time – are required to accurately calculate Adsorption Rate Constants [111]. Nevertheless, generally the greater a phage's Adsorption Rate Constant under *In Situ* conditions, and thereby Adsorption Rate, then the more suitable a phage will be for Phage Therapy purposes. For an essay on phage Adsorption Rate Constants, and theory, see Abedon [90,91,95].

Anti-Biofilm Activity

A utility of phages as antibacterial agents is their potential to eradicate bacterial biofilms. See Active Penetration as well as Extracellular Polymeric Substance (EPS) Depolymerase for further discussion, which respectively are Anti-Biofilm Activity as mediated directly by phage infections (see also Active Treatment—Locally Active Treatment) and Anti-Biofilm Activity as effected by phage-produced enzymes. See Abedon [113] for an especially ecological consideration of the phage potential to eradicate bacterial biofilms versus that potential by antibiotics. For summaries of the phage-treatment-of-biofilms literature, see also [11,77,78].

Appelmans Protocol

Technique used for Breeding Phages that can involve recombination between more than one phage type [114-122]. Note though that the method originally was an non-Plaquing approach to phage Titering [123].

Attachment

Attachment is the step in virion Adsorption which follows virion-bacterium Encounter, and which is dependent, in a probabilistic manner, on sufficient Adsorption Affinity. The Attachment step ultimately is not reversible for the attaching virion, and is followed in the course of a normal phage infection process by phage nucleic acid translocation into the bacterial cytoplasm [124]. Attachment thus is the last step of the Adsorption process as well as the first step of the actual infection process.

Attachment generally is dependent on specific interactions between virion proteins and bacterial envelope-associated macromolecules, the latter, i.e., phage Receptors [125,126]. Furthermore, it is the rate of Free Phage Attachment which is described by Adsorption Rate Constants, and successful Phage Therapy is absolutely dependent on Phage Particle Attachment to target bacteria.

Auto Dosing

Auto Dosing as a term is intended to contrast with standard clinician- or patient-mediated means of drug application. Auto Dosing in addition tends to contrast with a medicament being delivered from an extrinsic or external source. Instead, with Auto Dosing the bioactive substance is generated at least in part within the body. In the case of phages, this Auto Dosing is a consequence of *In Situ* phage replication. Ideally, for the

sake of successful Active Treatment, Auto Dosing also results in phage Population Growth, and this will occur given bacterial densities which exceed Proliferation Thresholds. Furthermore, from a pharmacokinetic perspective, Auto Dosing can be considered to be an aspect of Metabolism as phage replication involves chemical changes to the phage. It also can be described instead as 'self-dosing' or 'self-amplification' [27].

Active Treatments are highly dependent upon Auto Dosing whereas Passive Treatments by definition do not require Auto Dosing, but instead require only Bactericidal Infections. Auto Dosing also allows for increases in phage numbers to effective densities in precise association with target bacteria, thereby contributing to Phage Therapy efficacy (see Active Treatment—Locally Active Treatment). Auto dosing also can serve to compensate for inefficiencies in phage Penetration to Target Bacteria following standard dosing since with Auto Dosing fewer initial phages need reach populations of Target Bacteria. Auto dosing furthermore can result in body exposure to fewer phages should Target Bacteria not be present, thereby contributing, at least in principle, to Phage Therapy safety.

Autophage (Auto-Phage)

Autophage, or Auto-Phage, describes a bacterial virus Formulated Product which has been prepared specifically for an individual patient. It is not obvious from this definition, as derived based on verbiage on various phage therapy-associated websites, that these phages necessarily have been isolated against Target Bacteria obtained from the to-be-treated patient, versus phages that instead are obtained from a Phage Bank of previously isolated phages. Such 'custom' *isolation* nonetheless likely is or at least should be the case when speaking of Autophages [14], as I consider further in the subsection below. An Autophage thus should be contrasted with use in Phage Therapy of pre-defined phage Cocktails, and ideally should be contrasted as well with the obtaining for Phage Therapy purposes of already isolated phages from a Phage Bank. Thereby, contrast Cocktail (or Prêt-à-Porter) with Phage Bank (or Sur Mesure) with Autophage (also Sur Mesure). As noted, however, it is uncertain whether the Phage Bank approach is always excluded from advertised Autophage generation.

Steinman [127] provides little indication of whether an Autophage is isolated against a specific etiology versus simply grown on that host ("fabriqué au moyen des germes responsables de l'affection que l'on veut traiter"), but does note further that a problem with Autophages is that while they can be very effective against the Targeted Bacterial strain, the same phage may not (I interpret) be very effective against other strains (i.e., from p. 59, "mais il n'est pas préparé contre les cultures secondaires qui

pourraient se developer"). Delacost [128], on the other hand, seems to equate Autophage with Bred Phage (p. 553): "De plus, il ne provoque pas de résistance et, si son pouvoir diminue, il peut être à tous moments exalté par ré-entraînement au contact des germes infectants (autophage)."

Phage Isolation against a Patient's Etiology?

Kutter [52] indicates that (p. 265), "In problem cases, new phage specific to the patient's bacteria are occasionally isolated from sewage, amplified and sent to the hospital; these are called 'autophage'." Similarly, from Kvachadze *et al.* [129], p. 646, "In some cases when the approved Cocktails (commercial preparations) do not work *In Vitro* against the pathogen isolated from patient's samples, we isolate specific 'autophage' against [a] patient's specific bacteria and use these phages for treatment of the patient." I'm of the opinion, particularly in terms of the indicated time spans, that the description from Pirnay *et al.* [130] is also equivalent, p. 936: "Sometimes custom phage preparations are developed for a patient's infection (autophage), a procedure that usually takes a few days to weeks." Thus, these authors appear to equate Autophage with the concept of phage isolation specifically against a given patient's etiology and particularly for the sake of subsequently treating that patient, though as noted it is not certain that in all cases Autophages are also newly isolated phages.

Bacterial Half Life

Bacterial Half Life is how long it takes to reduce a bacterial population in number by one half [86]. This value can be predicted, and Bacterial Half Life therefore can be a useful metric toward understanding what phage densities may be sufficient to result in the timely eradication of Target Bacteria, i.e., what phage Titers may constitute Inundative Densities. Bacterial Half Life given exposure to phages, and ignoring bacterial replication, is in particular equal to -ln(0.5)/kP, where k is the Phage Adsorption Rate Constant and P is phage density, i.e., *In Situ* Titer. Certainly if many log-fold killing is desired over a given interval of time, then calculated Bacterial Half Lives should be supportive of desired rates of killing by a given expected *In Situ* phage Titer.

For example, given a phage Adsorption Rate Constant of 2.5×10^{-9} ml/min [100] and an *In Situ* phage Titer of 10^7/ml, then the expected Bacterial Half Life would be about 28 min, where -ln(0.5) = 0.69. In other words, after roughly one-half hour of phage exposure at this Titer, approximately half of the bacterial population would remain uninfected by phages, even assuming no Free Phage losses as well as, as noted, a lack of ongoing bacterial replication. A related but simpler as well as similar-magnitude metric (roughly 50% larger) is the bacterial 'mean free time', which is the average length

of time it takes until a bacterium becomes phage Adsorbed. This is equal simply to 1/*kP*. For an online Bacterial Half Life calculator, see Abedon [131]. See also an online decimal reduction time calculator [132].

Bactericidal Infection

A Bactericidal Infection by a phage results directly in the infected bacterium's death. This death can occur prior to phage-induced bacterial Lysis, and need not be associated with an otherwise successful phage infection. Especially, both Productive Infections and Abortive Infections are Bactericidal Infections. Bactericidal Infections are explicitly not associated with the establishment of successful Lysogenic Cycles, at least not immediately in terms of the initially Adsorbed bacterium. Bactericidal Infections also are prevented, despite phage Adsorption and infection, given successful expression by bacteria of restriction-modification systems against an infecting phage, or following successful anti-phage CRISPR-Cas display.

The proximate goal of Phage Therapy strategies should be for dosed phages to at least achieve Bactericidal Infections, i.e., as following Phage Particle Attachment to a Target Bacterium (see Lytic Infection—Purely Lytic Infection). Such infections should by definition be sufficient to achieve Passive Treatment, and, as noted, all Productive Infections by Lytic Phages are Bactericidal Infections. The transition of a Phage Particle to a Bactericidal Infection, i.e., as typically will occur given phage Adsorption to a bacterium that is found within its bactericidal Host Range [63], can be viewed pharmacokinetically as an aspect of Metabolism since it involves chemical changes associated with the infecting phage [87].

Bacteriophage Therapy

Bacteriophage Therapy, a.k.a., Phage Therapy, is the use especially of Phage Particles to combat bacterial infections as found particularly in either medical or veterinary contexts (dosing in principle can involve the application of phage-infected bacteria as well). This procedure can be viewed as a specific form of `.

Importantly, there is a preference by some authors to use the phrase 'Bacteriophage Therapy' over that of 'Phage Therapy' [133]. Therefore, when specifying keywords or otherwise searching for publications on this subject, it is best to use both terms, Bacteriophage Therapy along with Phage Therapy. For discussion of the distinctions between Bacteriophage Therapy and that of Phage-Mediated Biocontrol of Bacteria more generally, see Abedon [43].

Bacteriophage Insensitive Mutant (BIM)

A Bacteriophage Insensitive Mutant (BIM) is a bacterium which has mutated to phage Resistance. The term is common in the fermentation industry where it is desirous to protect bacteria from phage attack [134,135], that is, versus using phages to intentionally attack bacteria (the latter as is the case with Phage Therapy). In terms of protecting fermentation processes, a BIM may be isolated and, should it retain desirable fermentation characteristics, be used to replace starter bacteria which are sensitive to those phages that are currently prevalent in the fermentation environment. The term BIM nevertheless is useful for describing the phage-resistant bacterial mutants which can arise in the course of Phage Therapy.

Note that BIM does not stand for 'bacteriophage *induced* mutant' since, as we've known since Luria and Delbrück [136] and their fluctuation test, phages do not induce resistance mutations in otherwise phage-susceptible bacteria—at least except in terms of CRISPR-Cas systems, e.g., Medina-Aparicio *et al.* [137]. Rather, phages select for BIMs which are often present within bacterial populations prior to phage exposure. Note in addition that BIMs can differ phenotypically from their wild-type parents *not* just in terms of phage resistance, and this can include the displaying by bacterial pathogens of a reduced anti-host virulence [138] (see Virulence—Damaging to Bacteria…).

Biocontrol (Biological Control)

Biological Control, or Biocontrol, is the use of organisms or their products as antagonists to other, undesirable organisms. As such, Phage Therapy, with phages serving as antagonistic organisms, represents a form of biological control of unwanted bacteria [139,140]. Biological control using phages, i.e., Phage-Mediated Biocontrol of Bacteria, as a category, therefore is broader (arguably) than that of Phage Therapy. Phage Therapy thus is treatment of individual, bacteria-infected bodies especially toward preventing or curing disease in treated individuals – in other words treatment that is *therapeutic* in a medical sense – whereas Biological Control using phages includes the treatment of environments more broadly [43]. The latter can include phage treatment of foods post-harvest, of agricultural fields, or of environmental biofilms.

Bred Phage (Evolved Phage, Trained Phage, Phage Training)

Contrasting Engineered Phages, Bred Phages have been modified with classical genetical breeding approaches, that is, looking for and/or selecting for appropriate mutations, and then at least potentially crossing (recombining) phages so as to build up multiple mutations into a single lineage. Use of this specific term, Bred Phage, however

has been somewhat limited and Betts *et al.* [141] suggests instead 'Evolved phage' or 'Trained phage'. Notwithstanding what exactly to call them, historically it has been especially phage Host Range which has been modified in Bred Phages, particularly through serial transfer procedures in the presence of desired Target Bacterial strains [144]. Such phage breeding typically will result in adaptation of a phage lineage to a new host such that Productive Infections can occur. In addition, breeding can result in greater phage antibacterial Virulence (Virulent—Damaging to Bacteria as Virulent) that is against either an existing host or a diversity of similar hosts, e.g., [141,144-146]. "Phage training" is thought to be a promising approach to phage development for Phage Therapy [144]. See also Appelmans Protocol.

Serial Transfer-Based Phage Evolution

Serial transfer phage breeding is accomplished by *not* employing the pure culture technique of periodic population bottlenecking of a phage population to a single Plaque during phage stock propagation. Such serial transfer-based evolution, however, is likely to incorporate mutations into phage lineages which are in addition to mutations underlying those phenotypes which are being directly sought [147], with potentially unpredictable results. Consequently, a Bred Phage, or any organism subject to serial transfer, cannot be viewed as otherwise presumptively identical to its parent population. That is, useful mutations cannot be assumed to be present within genetic backgrounds which are isogenic to those of starting populations unless this has been confirmed through whole genome sequencing.

Burst

The term Burst is used synonymously with the concept especially of Lytic Release of Virion Particles from a phage-infected bacterium. Lytic Cycles thus end with a Burst of phages, and the number of phages released in a Burst is described as a Burst Size.

Burst Size

Burst Size refers to the number of new Phage Particles produced per individual phage-infected bacterium, and is the product of phage Productive Infections. Typically Burst Size is measured as an average group property such as in the course of One-Step Growth experiments. As such, Burst Size is applicable particularly to Lytic Phages, as typically used in Phage Therapy, rather than to chronically infecting phages (the latter such as phage M13). It is possible to also determine Burst Sizes on an individual infected-bacterium basis [148,149]; see also [150]. In either case, Burst Size here can be considered as an *absolute* Burst Size, absolute number of phages produced per phage-

infected bacterium, rather than the related but not identical concept of Effective Burst Size.

For Phage Therapy, Burst Size is relevant particularly to Active Treatment. The more new phages which a phage can produce per bacterium infected, *In Situ*, i.e., in the course of Auto Dosing, then the greater the potential for enough phages to be produced across environments to result in eradication of a majority of Targeted Bacteria in a timely manner, i.e., to achieve Inundative Densities of phages. Over smaller spatial scales it is possible also that larger phage Burst Sizes may be helpful toward combatting losses of virions in the course of, for example, phage Active Penetration into and subsequent elimination of Targeted Bacterial microcolonies within biofilms (see also Active Treatment—Locally Active Treatment) [75,86].

Clear Plaque

A Clear Plaque is one which lacks substantial turbidity. Turbidity within phage Plaques can be indicative of a failure of phages to lyse all of the Lawn bacteria found within the confines of a Plaque during Plaque development. Lack of Plaque clearness therefore can be a consequence of the presence of (i) Bacteriophage-Insensitive Mutants (BIMs), (ii) bacteria that have come to support Lysogenic Cycles (and therefore which display Superinfection Immunity upon Secondary Infection—Biomedical Sense), (iii) phage infections displaying greatly extended phage Latent Periods (e.g., such as Lysis inhibition in T-even-type phages, also as associated with Secondary Infection—Biomedical Sense), (iv) bacteria which are insufficiently metabolically active to support phage infection progress to the point of Lysis, or simply (v) because phages find it difficult to reach or adsorb some fraction of individual Lawn bacteria [151]. Adsorption difficulties could be due to poor virion Adsorption characteristics to Lawn bacteria under the plating conditions employed or instead because bacteria associated with individual microcolonies may physically ‘shade’ each other from phage Encounter [75].

Because Plaque turbidity can be indicative of deficiencies in the ability of specific phage types to kill specific bacterial types, it can be preferable to employ phages for Phage Therapy which produce Clear Plaques rather than turbid ones on Targeted Bacterial strains. A possible exception, however, is turbidity as due to Lysis inhibition [54,97,152] as that phenotype at least arguably does not represent a deficiency in phage anti-bacterial Virulence (Virulence—Damaging to Bacteria as Virulent). Note, though, that it can be important to reasonably well match *In Vitro* with *In Situ* conditions during Plaque assays to better assure a predictive power of Clear formation versus lack-of-Clear Plaques.

Clearance Threshold (Inundation Threshold, Minimum Bactericidal Concentration)

The phage Clearance Threshold is that *In Situ* Titer necessary to achieve successful Passive Treatment [83,84]. This contrasts with phage Inundative *Density* (which can be defined nearly equivalently) as the Clearance Threshold unlike Inundative Density has no explicit time component. The Clearance Threshold in addition is greater than the Inundation *Threshold* as the latter only defines that phage Titer that is *not* quite adequate to reduce bacterial densities. Indeed, explicitly in terms of phage Titers, Inundative Density > Clearance Threshold > Inundation Threshold, that is, these are the phage densities required to eliminate Target Bacteria over reasonable time frames, simply eliminate Target Bacteria but not necessarily over reasonable time frames, and only control bacterial Population Growth, respectively.

In all of these cases, an assumption is made, for the sake of both conceptual and calculation ease, that phage infection does not result in increases in phage densities at the moment in time that is being considered. Rather, these are descriptions of the impact of a given, existing *In Situ* phage density, whether generated by standard dosing or instead by Auto Dosing. The Clearance Threshold thus can be described as the minimum phage concentration necessary to eradicate a bacterial population given an absence of phage Productive Infection but resulting in Bactericidal Infections, that is, a Minimum Bactericidal Concentration. It is my opinion [153], however, that Killing Titer calculations, especially in combination with Bacterial Half Life calculations, can be more useful measures of the potential for a given phage *In Situ* Titer to eradicate bacterial populations than Clearance Thresholds.

Cocktail

Cocktails – as equivalent to Polyphage or Multiphage and contrasting Monophage – are phage Formulated Products containing more than one type of phage [146,154-168]. The utility of cocktails is that they can possess, due to the combined Host Ranges of the phages present, a broader antibacterial spectrum of activity than a Monophage Formulated Product. This means that cocktails can be better able to prevent the evolution of phage resistance *In Situ*. Cocktails also can be better able to address phage resistance as it can appear or evolve within human communities—'appear' here refers to newly problematic bacterial strains versus 'evolve' which refers to modifications of previously problematic bacterial strains, with the latter represented by, i.e., Bacteriophage Insensitive Mutants (BIMs). Lastly, Cocktails are better able to support Presumptive Treatments.

Prêt-à-Porter phage Formulated Products typically would be Cocktails. In principle Sur-Mesure products can be Cocktails as well. The latter, however, have less of a need to be Cocktails due to reduced requirements for either a broader spectrum of

activity or Presumptive Treatment abilities. That is, with Prêt-à-Porter the etiology has not necessarily been characterized prior to phage treatment whereas with Sur-Mesure in fact it has been priorly characterized, at least in terms of phage susceptibility. Note that various quantitative strategies have been developed for phage Cocktail optimization [158,160,169-174].

Community Resistance

Bacterial Resistance to phages that arises prior to the start of Phage Therapies. Contrast with both Treatment Resistance and phage Tolerance. See [28].

Confluent Lysis

To be confluent is to mix or run together, implying the existence of spatial structure, i.e., presence of impediments to mixing, but here impediments which are at least partially overcome. Confluent Lysis therefore is Lysis that runs together, particularly as observed during phage infection of bacteria growing in association with agar. This confluence occurs, in turn, when there are sufficient numbers of phages plated that Plaques run together during their formation, with indeed Confluent Lysis marked by a substantial absence of intact lawn bacteria on Petri dishes given phage plating. Though typically this confluence of Lysis will be seen as a consequence of inadvertent plating of too many phages, it also can be accomplished purposefully in the course of phage stock preparation using solid media rather than broth, i.e., the confluent plate lysate method [175].

Not Examples of Confluent Lysis

An isolated Plaque is not an example of Confluent Lysis, since with plaques Lysis is not being combined from more than one initial source, i.e., from more than one PFU. Confluent Lysis furthermore should not be equated with Lysis from Without as typically the Lysis itself, as seen with Confluent Lysis, is that which is observed at the end of a typical phage Lytic Cycle. i.e., as representing Lysis from within during Plaque formation (see Lysis). Local areas of clearing as can be seen during High-PFU Spotting technically also do not necessarily represent Confluent Lysis. Specifically, if sufficient numbers of phages are applied that subsequent phage Population Growth is *not* required for the formation of zones of inhibition of bacterial growth, then this is not a ‘confluence’ of Lysis, but instead simply multiple independent bacterial Lysis events. Nevertheless, unless in this latter case the phages employed can Bactericidally Infect but not Productively Infect, then it is reasonably likely that at least some phage population growth along with localized initiation of Plaque formation – and thus the ‘flowing

together' of immature plaques – may in fact occur, that is, resulting in some degree of Confluent Lysis.

Combination Therapy (Polytherapy)

Combination Therapy or Polytherapy refers to the use of more than one medicament, or procedure, per treatment of a disease [155]. If this is more than one phage used in combination, then generally the term Cocktail is used (equivalently, Polyphage or Multiphage). Though not necessarily easily achieved by phage Cocktails [176], at least among wild-type phages [177], Combination Therapies ideally will be associated with Synergistic interactions between components, though certainly additive-only interactions can be an acceptable outcome as well [178]. What needs to be avoided is where one component substantially nullifies the actions of another, that is, antagonistic combinations will tend to be problematic as this worsens overall efficacy relative to the impacts of individual components. In other words, even relatively small improvements given combinations can be worthwhile, but generally combinations working worse than the individual components are not helpful.

Of particular interest as a Combination Therapy, for Phage Therapy, is the potential to combine both phages and antibiotics within the same treatments [178]—see Chanishvili [179] for additional summary of the literature on phage-antibiotic Combination Therapy. See also, e.g., Oechslin *et al.* [180] and Valerio *et al.* [181]. Note that in Combination Therapy of phages with antibiotics, generally there is an expectation that antibiotics *might* be antagonistic to phage activity – resulting in reduced phage Performance/Infection Vigor particularly given use of bacteriostatic antibiotics – and this is rather than expectations that phages will be antagonistic to antibiotic activity. In addition, note the potential for synergism between phages in Phage Therapy with other phenomena, particularly with immune systems [12,182,183], and also with medical procedures such as debridement [11].

It is important to recognize in terms of synergistic, additive, or antagonistic interactions between components of Combination Therapies that not all aspects of phage Performance are essential for all Phage Therapy scenarios. Consider especially that phage Performance requirements will tend to be lower for Purely Passive Treatments versus Active Treatments. Thus, for Passive Treatment, combinations that negatively impact a phage's ability to reproduce, such as due to the action of bacteriostatic antibiotics, would be *not* detrimental to overall efficacy so long as a phage's ability to display Bactericidal Infections is retained. For Active Treatments, however, antibiotic interference with a phage's ability to produce new virions could be highly detrimental.

Cross Resistance

Cross Resistance refers to the potential for individual genetic components to reduce the susceptibility of an organism to two distinct antagonistic agents, e.g., multiple bacteriophages and/or antibiotics. By definition, this would represent a pleiotropic effect (one locus controlling two or more aspects of phenotype) and can be seen with any number of mechanisms of acquired Resistance. For phages, Cross Resistance is typically seen when two phages share a bacterial surface Receptor, one which otherwise, i.e., in the non-mutated form, would be used for virion Adsorption/Attachment.

Generally Cross Resistance to a combination of phage and antibiotic as based on mutations to bacterial-surface Receptors for phage Adsorption is not expected. That is, where one mutation or mechanism results simultaneously in Resistance to both entities. It is not inconceivable, however, that barriers to agent penetration to bacteria and/or the formation of more robust biofilms, for example, could give rise to such phage-antibiotic Cross Resistance. A phage Cross-Resistance avoider calculator can be found online at [169].

Crude Lysate

Crude Lysates are the direct products of phage stock preparation, having undergone minimal subsequent purification, e.g., no more than removal of larger debris and living bacteria through low-speed centrifugation, filtration, or chemical treatment (e.g., chloroform). Certainly with Crude Lysates, no effort toward phage 'extraction' from the medium has been undertaken. A Crude Lysate therefore contains numerous impurities including bacterial debris, bacterial toxins (e.g., endotoxin), other bacterial metabolic products, and what is left of the ingredients making up the original culture medium. The use of Crude Lysates for Phage Therapy purposes prior to more modern times, *sensu* Abedon [10], i.e., prior to roughly the mid-to-late 1990s, nevertheless appears to have been widespread [9,184], and indeed continues to be common among phage Formulated Products used clinically today.

Culture Lysis

Short for culture-wide phage-induced bacterial Lysis, Culture Lysis is as distinguished from the Lysis of individual bacteria. The Lysis of a culture by phages, however, is not necessarily equivalent to the Lysis of all bacteria within a culture but instead only, ideally for Phage Therapy, all of the phage-sensitive bacteria. The idea of

Culture Lysis is relevant particularly to *In Vitro* phage stock preparation [175] or *In Vitro* testing of phage antibacterial efficacy (see Virulent—Damaging to Bacteria).

Culture Lysis can be easily visualized and therefore can serve as a helpful marker of successful phage Population Growth and/or of bacterial elimination by phages. Culture Lysis in many cases also can be viewed as the broth equivalent of Confluent Lysis, where with Confluent Lysis one observes Culture Lysis or approximations of Culture Lysis instead with solid or semi-solid media. Equivalently, a localized Culture Lysis is seen within individual phage Plaques, and see too the consequences of successful Spot/Spotting—High-PFU Spotting.

Distribution (Pharmacokinetics)

Distribution, per pharmacokinetics, is movement of medicaments into tissues from out of systemic circulation. Thus, phage movement out of the blood, following systemic delivery, and into targeted organs, e.g., the prostate, would be an example of Distribution. With Phage Therapy, however, the more general term of 'Penetration' may be used instead of Distribution. In terms of pharmacokinetics, contrast Distribution with Absorption.

Drop Plaque Method

See Spot/Spotting—Low-PFU Spotting.

Eclipse (Eclipse Period)

The Eclipse, or Eclipse Period, is the span of time between phage virion Adsorption and the presence within the phage-infected bacterium of the first otherwise mature progeny phage virion [185,186]. This span has important bearing on the phage Burst Size since intracellular phage progeny only accumulate toward that Burst Size once the Eclipse Period has ended. Thus, the first period of a phage Latent Period, known as the Eclipse, by definition does not directly contribute to intracellular phage virion progeny accumulation. What occurs molecular during the Eclipse, however, presumably has some bearing on *rates* of phage virion-progeny intracellular accumulation following the Eclipse.

Note that it is possible for authors to use Eclipse Period when what they mean instead is Latent Period, so be aware of usage. Particularly, there are few contexts within Phage Therapy in which Eclipse Period is sufficiently relevant for use of the term,

so the possibility of mistaken usage should be easy to spot. Another relevant point is that the Eclipse Period is not followed by the phage Rise, but instead it is the Latent Period that is followed by the phage Rise. Phage infections therefore take place in the following sequence: Adsorption (thus beginning the Latent Period) is followed by Eclipse Period, is followed by a post Eclipse Period during which intracellular phage progeny accumulate intracellularly (*not* called a Rise), and this is followed by the end of the Latent Period, and with latter associated with virion Release, which for Lytic Phages occurs via Lysis.

Effective Burst Size

Effective Burst Size, as more generally can be described as a reproductive ratio [187], is the number of phages Released per Burst which survive to produce especially Productive Infections of their own [27,104,182,188-190]. For further discussion, see Proliferation Threshold, which is that bacterial density which can support an Effective Burst Size that is equal to one. See also Secondary Infection—Epidemiological Sense, where Effective Burst Size can be viewed as more or less equivalent to the number of 'Secondary Infections' generated per Primary Infection (with those terms both defined epidemiologically).

For Active Treatment to be efficacious, then Effective Burst Sizes must be greater than one. Depending on a combination of the densities of Target Bacteria present along with what defines a phage's Inundative Density (and how quickly treated bacterial infections need to be brought under control), then Effective Burst Sizes potentially must be much greater than one for Active Treatments to be successful. For example, this could be ten-fold increases in numbers of subsequently phage-Productively Infected bacteria per bacterium infected, which would be an Effective Burst Size of 10.

Alternatively, Gadagkar and Gopinathan [191] as well as Patel and Rao [192] defined Effective Burst Size as the ratio of Burst Size to number of phages which have Adsorbed per bacterium. It is important with such usage, however, that measures indeed are made per bacterium rather than simply per colony-forming unit (CFU), as the latter instead can consist of multiple bacteria, which potentially can result in more than one actual Burst per CFU [27,73].

Efficiency of Center of Infection (ECOI)

Efficiency of Center of Infection (ECOI) determinations are Plaquing-based means of assessing phage viability during infection of a given host bacterial strain [56,193,194]. With ECOI determinations, phages are plated as preadsorbed phage-infected bacteria

rather than as Free Phages, using an otherwise permissive strain of indicator bacteria – that is, one able to support Plaque formation with relatively high efficiencies – and also otherwise permissive plating conditions. In this manner, only the first round of phage infection during Plaque formation is selective. Successful production of phage progeny, i.e., a Productive Infection during that first round, therefore is highly likely to ultimately produce a Plaque. ECOI determinations consequently can be a conceptually less complex means of determining a phage's productive Host Range than Efficiency of Plating (EOP) determinations, and this is because Plaque formation for ECOI determinations is more likely, given an initial phage-Productive Infection, than can be the case with EOP determinations.

Because for successful ECOI determination Free Phages cannot be plated, ECOI assays are more technically demanding than EOP determinations. EOP determinations, in turn, are more technically demanding than High-PFU Spotting. Thus, in terms of experimental ease, High-PFU Spotting is easier than EOP determinations, which are easier than ECOI determinations, and ECOI assays in turn can be easier to perform than broth-based phage characterizations such as One-Step Growth experiments. Furthermore, less phage infection Performance is required to achieve a positive result for ECOI determinations – only a single phage need be produced during the first round of replication – than is the case for EOP determinations, where typically it is thought that at least roughly ten phages (actual Burst Size) must be produced per phage infection to produce a Plaque [195]. In terms of phage infection Performance, however, note that at least in principle phages need display only Bactericidal Infections to produce Spots (Spot/Spotting—High-PFU Spotting).

Preadsorption

Note that preadsorption as the term is employed here (previous paragraph) refers to a prolonged mixing of phages with bacteria in liquid media prior to the plating process, that is, so as to promote irreversible phage Adsorption [60] and thereby Plaque formation from already phage-infected bacteria. An alternative meaning of the term preadsorption, however, is provided by the ACLAME Phage Onolology [60]: "Any process by which a phage loosely binds to its host surface and scans it for receptors with its fibers, spikes or a baseplate component." This latter perspective is synonymous with reversible Adsorption [92]. In any case, following such preadsorption (first definition), with an ECOI assay it is essential to physically separate phage-infected bacteria from Free Phages prior to plating because Free Phage plating otherwise would result directly in Plaque-formation false positives.

Efficiency of Plating (EOP)

With Efficiency of Plating (EOP) [47,100,196,197], plating refers to Plaquing and efficiency refers to the fraction of Plaques which form in comparison to some ideal for the phage being characterized. That ideal may be absolute in terms of total number of Virion Particles plated, with the latter numbers determined microscopically (i.e., typically electron microscopically). Alternatively, that ideal may be relative to the number of Plaques produced under more optimized conditions. As based on this latter approach, typically EOP experiments are performed as a means of characterizing a phage's Host Range, with lower EOPs, holding plating conditions otherwise constant, indicative of an indicator bacterium host which is less central to a phage's Host Range [196,197].

Generally EOP determinations should be viewed as a more robust and certainly quantitative means of phage Host Range determination than Spotting with high phage Titers (Spot/Spotting—High-PFU Spotting). EOP also supplies different information from Efficiency of Center of Infection (ECOI) determinations [56] or, indeed, from broth-based determinations of phage viability. True positive results following High-PFU Spotting specifically requires only Bactericidal Infections, i.e., the killing of lawn bacteria very early during lawn development, while ECOI-assay true positives require only a single Productive Infection of the bacterial strain in question. Plaque formation during EOP determinations by contrast requires that many successfully Productive Infections occur in both series and parallel. What exactly determines a given phage's plating efficiency nevertheless generally tends to be poorly characterized. See the following subsection as well as further more general discussions of the complexities associated with phage Plaquing [56,151,198-200].

Reasons for Lower Efficiencies of Plating

Plaques which form given especially lower EOPs (e.g., $<10^{-4}$) may represent simply phage Host-Range mutants, or instead epigenetic phage modifications in terms of overcoming restriction-modification systems. With higher EOPs, a lower Plaque forming ability, i.e., less than 1.0, could be a consequence instead of what may be referred to as a lower phage Infection Vigor, i.e., low Burst Size or extended Latent Period. Indeed, it is possible to show statistically that within a given stock fewer phages may successfully form Plaques than can Productively Infect bacteria in broth [201]. Alternatively, in this latter, higher EOP case, not all phage infections of individual bacteria, i.e., especially those potentially initiating Plaques, may be Productive Infections (e.g., see Abortive Infection).

Encounter

Physical interaction between a Free Phage and the surface of a bacterium. If the bacterium is found within a phage's Host Range, then Attachment may follow. This is the second step of the overall phage Adsorption process, consisting of (0) Release (not a part of Adsorption), (1) diffusion, (2) Encounter, (3) Attachment, and then (4) uptake of the phage nucleic acid. Encounter rates should increase as a function of the size of the targeted bacterium [90] and indeed the size of the clonal arrangement, cluster, or microcolony [73] that the bacterium is found in, though the latter is not with a specific, individual cell. For more on what can affect rates of Encounter see [91] . See also Adsorption Affinity.

Endolysin

An Endolysin is a phage-produced and phage-encoded enzyme that digests and thereby weakens bacterial cell walls, to the point of effecting an osmotic lysis under hypoosmotic conditions. Most phages produce endolysins as part of their mechanism of so-called lysis from within, that is, normal phage-induced Lysis of bacterial cells as seen at the end of phage Latent Periods. Alternatively, virion-associated endolysins, so-called ecotolysins such as gene product 5 of phage T4 [202], can digest cell walls during virion Adsorption and can result in what is known as a Lysis from Without.

It is possible to purify Endolysins and use them as antibacterial agents [203-210]. This antibacterial action also is described as effecting a Lysis from Without, as these purified Endolysins in this case are applied to and otherwise interact with bacteria extracellularly, though this nevertheless is distinct from the Lysis from Without which can be effected by whole phage virions. Such purified, 'Lysis from Without'-effecting Endolysins represent a key category of phage-derived Enzybiotics.

Engineered Phages

Contrasting Bred Phages, an Engineered Phage has been modified either strictly phenotypically or, more often, via genetic engineering in order to take on new properties [21,163,166,177,211-220]. Often what especially is envisaged as being modified in Engineered Phages, as to be used for Phage Therapy, is phage Host Range, e.g., such as by engineering of tail fiber genetic loci. Phage-immune system interactions may be modified as well, or Phage Particles may be adhered to surfaces, etc. An issue with genetic engineering of therapeutic phages, however, is that these phages then represent genetically modified organisms, thereby potentially negatively impacting the process of their gaining regulatory approval as medicaments.

Enzybiotic

'Enzybiotic' [221] combines the terms enzyme and antibiotic, with an enzybiotic thereby an enzyme with antimicrobial properties. Phage-derived Enzybiotics [42,202,204,222-225] most prominently include purified Endolysins, but also can include purified phage-derived Extracellular Polymeric Substance (EPS) Depolymerases.

Excretion (Pharmacokinetics)

Excretion, in a pharmacokinetic sense, is movement of a medicament from inside of the body to outside of the body, with the medicament in the process remaining chemically in a more or less intact form. Most prominently this is movement mediated by the kidneys or instead by the Liver into the gastrointestinal tract. For Phage Therapy, excretion is most relevant to the extent that it can result in the transport of Phage Particles from systemic circulation into urine for the sake of treatment of urinary tract infections [226-232].

Extracellular Polymeric Substance Depolymerase (EPS Depolymerase)

An Extracellular Polymeric Substance Depolymerase is an enzyme that is able to hydrolyze, that is, break down bacterial glycocalyx. This can include capsules, slime layers, or, most notably, biofilm extracellular polymeric substance (EPS), i.e., biofilm matrix material. Numerous phages have been found to encode EPS Depolymerases [233]. EPS Depolymerases can aid phages in reaching bacterial surfaces during Adsorption processes, and this is particularly so to the extent that these enzymes are virion associated [199], with EPS depolymerases often consisting of virion proteins [233]. EPS Depolymerases may also aid Phage Particles as they disperse away from biofilms, which in principle could be a function of both virion-associated and soluble depolymerase enzymes produced by phage-infected bacteria [199].

EPS Depolymerases, in terms of Phage Therapy, most notably have the potential to aid in the dispersion of bacterial biofilms [42,234]. Furthermore, EPS Depolymerases can be supplied to bacteria in a purified form independent of their encoding phages [235], i.e., as Enzybiotics. The principle caveat with EPS Depolymerases, however, is their potential for high specificity, which can result in excessively narrow spectra of activity. In addition, it is not obvious that phage encoding of EPS Depolymerases necessarily or at least consistently supplies substantial real-world improvement to efficacy, i.e., such as clinically.

Formulated Product

A Formulated Product consists of a combination of active and other ingredients with which one doses, such as during Phage Therapy. Note that it is important during reporting on Phage Therapy to be precise in terms of the final, within-dose Titers of all phage types which have been included in Formulated Products, i.e., phage A is present at Titer X, phage B is present at Titer Y, phage C is present at Titer Z, etc. The use of alternative approaches to describing these amounts, that is, often can be ambiguous, making experiment replication or interpretation difficult or even impossible [36].

Free Phage

A Free Phage is a virion that is *not* found within its parental phage-infected bacterium nor has subsequently Adsorbed to a bacterium. It is the process of virion assembly (maturation) in combination with subsequent virion Release (e.g., Lysis) which is responsible for the generation of Free Phages. Generally it is Free Phages which are supplied as the active ingredient of phage Formulated Products that are destined for use as antibacterial phage therapeutics. Absorption, Adsorption, Adsorption Affinity, Attachment, Adsorption Rate Constants, Distribution, and Excretion all describe the actions, movement, or properties of Free Phages, and Formulated Product stability is usually measured in terms of the continued viability of Free Phages. One can also speak of the half life of Free Phages in the presence of susceptible bacteria [112]. Densities of Free Phages generally should be described in terms of phage Titers.

Complications on Experimental Free Phage Assessment

When mixed with bacteria such as during One-Step Growth experiments, or during phage therapy, it can be relevant to recognize that not all Plaque-forming units (PFUs) may be the result of plating Free Phages. This is particularly so unless efforts are made to plate only Free Phages, e.g., such as by treating cultures with chloroform (which typically will kill bacteria including phage-infected bacteria) or separating free phages from phage-infected bacteria via filtration or centrifugation. The concept of 'infective center' thus may be used instead to describe both phage-infected bacteria and Free Phages, which is useful especially when efforts to separate Free Phages from phage-infected bacteria have *not* been made. The concept of PFU thus is not identical to that of Free Phage.

Note that artificial lysis of phage-infected bacteria, such as via chloroform treatment but also potentially as a consequence of rough handing of cultures, can result as well in the Release of additional Free Phages from these bacteria [185]. Thus, care must be taken when striving to explicitly assess Free Phage counts *In Situ* during phage

therapy experiments, that is, to avoid either plating or artificially lysing phage-infected bacteria. In addition, Free Phages may adsorb bacteria following disruption of the spatial structure of environments as done for the sake of phage or bacterial enumeration, thereby resulting not just in enumeration-associated losses of uninfected bacteria [236-238] but in losses of Free Phages as well.

Halo

A Halo is a region that is found around phage Plaques or Spots, consisting of an area of bacterial Lawn that has been partially reduced in turbidity [239-242]. Halos typically are caused by the production, by phages, of Extracellular Polymeric Substance (EPS) Depolymerase, which digest Lawn-bacterium-associated EPS. Halos can continue to expand even following otherwise cessation of Plaque growth, and can continue to increase in size even during refrigeration as Halo formation is due to a simple enzymatically catalyzed reaction.

Generally claims that a phage possesses EPS Depolymerases which are active against specific bacterial hosts should not be made unless production of a Plaque Halo for that phage has in fact been observed. Note also that in Gram-positive bacteria, notably as seen with *Streptococcus lactis*, halos also have been reported to form as a consequence of actions attributed instead to a Lysin [243,244]. Furthermore, with Gram-negative hosts, Halos can potentially result as well from degradation of lipopolysaccharide carbohydrates [245].

High Molecular Weight Bacteriocin (Phage Tail-Like Bacteriocin)

High Molecular Weight Bacteriocins, i.e., Phage Tail-Like Bacteriocins [246], are bacteria-produced antibacterial agents that are both quite specific in their antibacterial activity (as bacteriocins) and which morphologically resemble the tails of Phage Particles. As such, they may be considered to be phage-like as potential therapeutic agents, though given their lack of genomes, Tail-Like Bacteriocins are capable only of Purely Passive Treatment.

The term Tailocin has been suggested as a simpler synonym [247,248]. More traditional are the terms F-type bacteriocin and R-type bacteriocin, which typically are named after the specific bacteria involved, particularly but not exclusively with F-type and R-type pyocins associated with *Pseudomonas aeruginosa*. These are *Siphoviridae*-related (F-type) and *Myoviridae*-related (R-type) High Molecular Weight Bacteriocins, respectively.

Host Range (Phage Specificity)

Host Range, a.k.a., Phage Specificity, refers to the types of bacteria (species, strains, etc.) that a phage is capable of interacting with in a specific manner [63,197]. For Phage Therapy purposes, this manner typically would be in terms of the ability of the phage to kill Targeted Bacteria (bactericidal Host Range; see Bactericidal Infection) and/or in terms of a phage's ability to produce new virions while infecting Targeted Bacteria (productive Host Range; see Productive Infection). In addition there is a phage's Transductive Host Range, that is, what bacteria a phage may be capable of delivering bacterial DNA to, even if that phage is not necessarily otherwise able to Bactericidally or Productively Infect the recipient bacterium.

Bactericidal Host Range is relevant especially to Passive Treatment while productive Host Range is relevant especially to Active Treatment. In addition, gradations may be present, i.e., such that, for example, different degrees of productivity or bactericidal activity by a given phage may exist for different host strains, as well as in different contexts, or in terms of different measurements. An example would be in terms of phage Burst Size for the productive Host Range, e.g., with a somewhat smaller Burst Size suggesting that a given bacterial strain is less central to a phage's productive Host Range than one upon which Burst Sizes are larger (see also Performance as well as Infection Vigor).

In terms of assays, Spotting using high phage numbers (Spot/Spotting—High-PFU Spotting) can provide a first-level approximation of bactericidal Host Range, though do be concerned about false-positive results (i.e., spot formation despite a lack of phage virion-induced bacterial killing). Plaque formation can provide a good indication of productive Host Range, though do be concerned about false negatives (see also Spot/Spotting—Low-PFU Spotting), i.e., failures to produce Plaques despite Productive Infections (see Efficiency of Plating).

For Phage Therapy, note that there is an overlap between the concept of Host Range and the pharmacological concept of spectrum of activity. For phage Cocktails, spectrum of activity is the collective Host Range of the phages present.

Immunity (Homoimmunity, Superinfection Immunity)

Also known as Homoimmunity, or Superinfection Immunity, Immunity as this term is typically applied to phages specifically describes a mechanism expressed by Prophages which has the effect of preventing similar phages from successfully infecting bacterial lysogens. The existence of Immunity is one reason that Temperate phages tend to be avoided for Phage Therapy purposes, since a certain fraction of bacterial infections

by a Temperate therapeutic phage would result in conversion of the Targeted Bacterium into one which is refractory to eradication by that same phage type. That is, those Target Bacteria which come to display both Lysogenic Cycles and Superinfection Immunity following infection by these phages.

Heteroimmunity versus Homoimmunity

Immunity as expressed by a given phage type tends to be effective against only a narrow range of potentially superinfecting phages, i.e., against phages that are equivalent to the expressing (primary) phage or instead against phages which are closely related in terms of lysogeny-maintaining repressor proteins. In either case, Immunity is against phages which are Homoimmune. Note also the concept of heteroimmunity, which describes the immunity of wild-type Temperate phages that are able to avoid the immunity expressed by Prophages of other immunity types. That is, if Temperate phage A is able to routinely successfully infect a lysogen of Temperate phage B, then phages A and B would be described as heteroimmune, and particularly so to the extent that phage B equivalently was able to superinfect despite the presence of Prophage A (but not able to superinfect given the presence of Prophage B). By contrast, if Prophage B were able to display Immunity against Temperate phage C, then phages B and C would be said to be Homoimmune, though phages B and C need not necessarily be otherwise closely genetically related. See also Virulent (—Temperate Phage Mutant as Virulent), which describes Temperate phage mutants that are able to overcome Homoimmunity.

Limitations on Immunity as a Phage Term

Note that Immunity and exclusion, the latter as in superinfection exclusion, are *not* identical concepts. Instead, Immunity is an intracellular process which is associated with expression of Prophage repressor genes [249,250], whereas exclusion is a process which acts at the bacterial cell envelope and which serves to prevent phage nucleic acid uptake especially into already phage-infected bacteria [97]. Therefore, these two terms should not be used interchangeably. In either case, these nevertheless are mechanisms expressed by Primary Infections which serve to inhibit Secondary Infections, with both of these latter terms (Primary and Secondary) being used here in a Biomedical Sense (see Secondary Infection—Biomedical Sense). Immunity also should not be used to describe more generally various bacterial anti-phage mechanisms [65] such as restriction-modification, CRISPR-Cas, or Abortive Infection systems.

In Situ

In Situ, from Latin, means ‘in place’. For Phage Therapy, as observed within the context of a phage-treated environment, *In Situ* refers particularly to being present

within less-simplified model systems or during actual, e.g., clinical procedures. Thus, it is desirable for phages to retain their *In Vitro* properties *In Situ*, and *vice versa*. The term *In Situ*, however, can also be used to describe circumstances within any treated environment, including simplified model systems, with knowledge of context typically required to infer meaning. For instance, in considering just *In Vitro* experiments, *In Situ* still may be used to refer to what is going on within those experiments, e.g., what is happening within the test tube.

For the treatment of environments which are not within other organisms, i.e., which are not *In Vivo*, then *In Situ* is the relevant descriptor, e.g., *In Situ* within a phage-treated pond. Phage Titers as measured *In Situ* thus would be phage concentrations as found within a treated environment such as following dosing, whether this is within an animal, or within a pond, etc. Note further that Phage Therapy efficacy will tend to be highly dependent on *In Situ* phage Titers, which generally must attain Inundative Densities, at least locally, for antibacterial therapy to be effective.

In Vitro

In Vitro, from Latin, means 'in glass'. For Phage Therapy, *In Vitro* is as observed within simplified models, ones which especially are not subsets of larger environments. *In Vitro* also, and equivalently, is as not found within other organisms such as animals. Testing of phages within broth cultures, using Petri dishes, or against biofilms grown in the laboratory are all examples of *In Vitro* analyses.

Typically, in Phage Therapy, at least some *In Vitro* data is gathered before turning to *In Vivo* or *In Situ* testing. Indeed, given the costs as well as ethical issues associated especially with *In Vivo* testing, it can be helpful to first place some emphasis on *In Vitro* analyses – such as determination under realistic conditions of phage Adsorption Rate Constants , Latent Periods, Burst Sizes, ability to produce Clear Plaques, and Host Range, as well as undertaking bioinformatic analyses [39] – prior to performing more involved *In Vivo* or *In Situ* studies.

Use in Phage Biology (not Phage Therapy)

For analyses of phage biology more generally, note that simplified systems, but ones which nevertheless still employ intact bacteria as hosts, may be described as *In Vivo* rather than as *In Vitro*. Here, *In Vivo* refers to phages being studied in the course of being found inside of living bacteria. Biochemical analyses of phage biology, when focusing specifically on what can occur within cell-free extracts, on the other hand, would be described as *in vitro*. The concept of *In Vitro* thus can be context dependent with phages. Focus that is particularly on bacteria rather than on larger environments

thereby often is described as *In Vitro* for Phage Therapy, such as phage treatment of bacterial broth cultures within flasks or microtiter plates, while focus on larger, more complex environments, such as treatment of animals or ponds, instead will tend to be described in terms of *In Vivo* or *In Situ*, but especially *in vitro* and *In Vivo* can have other meanings in the context of phage biochemical analysis.

In Vivo

In Vivo, from Latin, means 'in a living thing'. *In Vivo* generally is applicable to Phage Therapy that is occurring within other organisms, e.g., such as within animals or plants, i.e., other than solely in association with phage-Targeted Bacteria as the living thing. Phage application to bacteria as found within test tubes, Petri dishes, or laboratory grown biofilms thus normally should *not* be described as taking place *In Vivo*. In a non-Phage Therapy context, however, *in vivo* certainly can and should include phage infections of bacteria more generally (see *In Vitro*—Use in Phage Biology… for broader discussion). With Phage Therapy, especially of animals including of humans, *In Vivo* may be used synonymously with *In Situ*, though context can still be important toward interpreting meaning.

In Vivo *Referring to Animal Testing*

More narrowly, it is possible to equate *In Vivo* studies especially with those experiments which consist of other than *In Vitro*, pre-clinical-type testing, e.g., animal testing. Standard Phage Therapy development such as for treatment of humans thus may be viewed as progressing, ideally, from *In Vitro* studies (i.e., basic phage characterization) to *In Vivo* studies (i.e., animal testing) to clinical testing and trials, e.g., *In Situ* studies [251]. The term *In Vivo* nevertheless, and more broadly, may be used to describe as well the context of actual clinical treatments, e.g., 'The phage therapy efficacy was tested *in vivo*, within the patient, with periodic *In Situ* monitoring of phage Titer within serum.'

Infection Vigor

Infection Vigor refers especially to levels of phage Burst Size along with durations of phage Latent Periods, with lower Infection Vigor associated especially with smaller Burst Sizes or longer Latent Periods. The term was coined toward considering how phage infection Performance could impact phage Efficiency of Plating, thereby potentially resulting in Abortive Infection-like outcomes. That is, to consider circumstances in which a phage's low Efficiency of Plating may be for reasons other than due to simply a phage's failure to produce any progeny at all [63]. The assumption is

that especially low phage Burst Sizes, e.g., less than 10 [195], or particularly long Latent Periods can also result in a reduced phage potential to efficiently form Plaques.

A phage displaying higher levels of Infection Vigor – reasonably large Burst Sizes in combination with reasonable short Latent Periods, thereby making such a phage likely to possess relatively high Efficiencies of Plating – would be potentially useful toward Active Treatment of the associated bacterial strain. Phages having low Infection Vigor would tend to be less likely to display relatively high Efficiencies of Plating, and also likely would be less useful for Active Treatment, again against the tested bacterial strain. Given adequate Adsorption Rates along with high likelihoods of Bactericidal Infection, however, then such low Infection Vigor phages nevertheless may still be adequate for Passive Treatment, as *In Situ* phage Population Growth in that case by definition is not necessary.

Burst Size-Latent Period Correlations

Note that an occurrence of larger Burst Sizes in combination with shorter Latent Periods, i.e., as defining higher Infection Vigor, is not a contradiction. Especially in terms of Infection Vigor, that is, these are physiological issues [252,253] rather than ones of between-infection variation [149] or evolutionary tradeoffs [254-256]. It is especially these latter concepts, however – that of longer Latent Periods inherently supporting larger Burst Sizes under otherwise constant physiological conditions – which tend to be more often considered in the literature, hence the potential for confusion. Thus, somewhat effectively infecting phages, i.e., ones displaying reasonably high infection Performance, will in many cases tend to display both relatively short Latent Periods and relatively large Burst Sizes, even though were these same phages to mutationally display longer Latent Periods, infection physiology otherwise held constant, then they would also display larger Burst Sizes.

Inundation Therapy

Equivalent to Passive Treatment, or therapy [83], Inundation Therapy is dosing with sufficient numbers of phages to achieve desired levels of bacterial eradication without depending on *In Situ* phage Population Growth, i.e., without requiring Auto Dosing. Such inundation may be accomplished given sustained *In Situ* phage Titers of roughly 10^8/ml (see Inundative Density). Thus, under circumstances in which bacteria are present at insufficient densities within environments to support Active Treatment, i.e., when bacteria are present within Numerical Refuges, it should be assumed that approximately 10^8 phages per ml, as explicitly applied to a treated volume, may be required to result in adequate bacteria-killing efficacy, and even more phages, per dose,

if these phages are to be diluted *In Situ* within existing volumes (e.g., the gastrointestinal tract). On the other hand, with non-Inundation Therapy, i.e., Active Treatment, such phage Titers instead may be achieved via *In Situ* phage Population Growth.

Multiplicity of 10 and Complications

Attainment of a Multiplicity of Infection (MOI_{actual}) of 10, or more, is generally considered also to be sufficient to approximate such inundation [257]. This number, however, is to a degree dependent on starting bacterial numbers. Particularly, it is less true for either very low or very high bacterial numbers since the former have fewer bacteria which need to be killed, thereby requiring fewer Adsorbed phages per bacterium to eradicate a population, while the latter have more bacteria to be killed, thereby requiring more Adsorbed phages per bacterium to achieve equivalent post-treatment numbers of remaining bacteria. For example, this could be killing 100 (10^2) bacteria versus killing 100 billion (10^{11}) bacteria, whereas as an MOI_{actual} of 10 results in roughly 20,000-fold bacterial killing (~10^5). In any case, note that this is the number of *Adsorbed* phages per bacterium, i.e., Multiplicity of Adsorption (= MOI_{actual}), rather than the number of phages simply added to bacteria (MOI_{input}). Such levels of phage Adsorption nevertheless should be relatively easily accomplished given sustained *In Situ* phage Titers of roughly 10^8/ml, though higher phage Titers may be required if Target Bacteria are difficult to reach or Adsorb.

Minimum Inhibitory Concentration

The Inundation Threshold is the minimum *In Situ* phage Titer required to control, but not to eliminate a bacterial population. The Inundation Threshold thus can also be viewed as a phage MIC, that is, minimum inhibitory concentration [153,258]. Like Killing Titer and Bacterial Half Life determinations, Inundation Threshold calculation therefore can be useful as a means of estimating whether phage densities *In Situ* may be sufficient to control versus not control populations of Target Bacteria. One must be able to reasonably approximate rates of bacterial replication in the absence of phages to calculate the Inundation Threshold, however, as well as determine the phage Adsorption Rate Constant.

Inundative Density

Inundative Density refers to sufficient phage concentrations, within an environment, i.e., *In Situ*, to result in sought degrees of bacterial eradication over reasonable, that is, preferred spans of time. Note that this concept to the best of my knowledge does not otherwise possess a name, hence it's inclusion here as Inundative

Density [86], though 'adequate *In Situ* phage Titer' might be used as a synonym. A phage Inundative Density may be achieved through some combination of adequate dosing and sufficient *In Situ* phage Population Growth. Note however that the latter itself is expected to introduce delays in terms of impact on Target Bacteria, and also requires sufficient densities of Target Bacteria be present within treated environments to support sufficient increases in phage numbers. Consequently, an Inundative Density is most readily conceptualized in terms of Passive Treatments rather than Active treatment, though nevertheless must be reached in the course of Active Treatment as well to result in satisfactory bacterial killing over reasonable time frames. An online Inundative Density calculator can be found at [259].

Titers of 10^8 Phages/ml as Inundative

By way of example, an Inundative Density could be sufficient *In Situ* phage numbers to result within 100 minutes after phage dosing in a Multiplicity of Infection (MOI_{actual}) of 10 or more (see Poisson Distribution as well as Inundative Therapy for the meaning of $MOI_{actual} = 10$). As MOI_{actual} can be predicted as Pkt [35], where P is the phage *In Situ* Titer, k is the Phage Adsorption Rate Constant, and t is the duration of phage Adsorption, then rearranging we have $P = 10/kt$, where here P would be the phage Inundative Density. Setting k, for example, equal to 2.5×10^{-9} ml^{-1} min^{-1} [100], and t to the noted 100 min, then P as Inundative Density is equal to 4×10^7 phages/ml, with the 100 min starting at the point that this *In Situ* Titer is reached.

Rounding up, for the sake of being conservative in terms of achieving bacteria-killing efficacy, then this would be 10^8 phages/ml as an Inundative Density. Thus, as I and others have argued elsewhere [61,133,260], for Phage Therapy generally, an *In Situ* Titer of approximately 10^8 phages/ml should be sought—whether this Titer is achieved only through standard dosing approaches, and thereby giving rise to Purely Passive Treatment (a.k.a., Inundation Therapy), or instead is achieved via Auto Dosing in the course of Active treatment. Successful treatment in terms of levels of bacteria killing over a given, desired time period requires in other words an achievement, by some means, of *In Situ* phage Titers that by definition (here) are equal to or greater than Inundative Densities. Furthermore, note that generally Inundative Densities will be greater than Inundative Thresholds and indeed also greater than Clearance Thresholds.

Killing Titer

Killing Titer determinations are a means of assessing the bacteria-killing potential of phage populations. This potential is measured in terms of starting numbers of bactericidal Virus Particles. This includes, for Killing Titers determinations, even phages

which are not capable of replicating, e.g., such as due to prior ultraviolet irradiation, or instead because they are Engineered Phages which have been modified so as to not lyse infected bacterial hosts [27,261]. The Killer Titer procedure takes advantage of assumptions that Phage Particles adsorb to Target Bacteria over Poisson Distributions. The fraction of not phage-Adsorbed and thereby not-killed bacteria thereby is expected to equal e^{-M}, where *M* is the phage Multiplicity of Infection (MOI_{actual}). See Abedon [262] for further discussion. For an online Killing Titer calculator, see [263].

Determining Killing Titers

In the course of *In Vitro* Killing Titer determinations, phages are Adsorbed to bacteria to some approximation of completion, i.e., such that Free Phages are depleted in number to roughly zero. The number of viable bacteria that were present prior to phage Adsorption is then compared with the post-phage-Adsorption number. The ratio of post-to-pre Adsorption ("fraction", below) is expected to be equal to the as noted e^{-M}. Bacteria otherwise are assumed to neither replicate over the course of exposure to Phage Particles nor be lost for reasons other than due to phage Adsorption.

Emphasizing the calculations:

e^{-M} = [fraction of viable bacteria remaining post phage adsorption] = ["fraction"].

Therefore, with *M* standing for Multiplicity of Infection (MOI_{actual}),

M = -ln[fraction of viable bacteria remaining post adsorption] = -ln["fraction"].

For Killing Titer (*K*), with density defined, e.g., in per ml units,

K = *M* × [density of viable bacteria present prior to phage adsorption],

and thus, with rearranging,

K = -ln["fraction"] × [density of viable bacteria present prior to phage adsorption],

In words, Killing Titer is equal to the opposite of the natural log (ln) of ratio of bacteria remaining to that number present prior to phage application, multiplied by the starting number of bacteria. In other words,

K = [number of adsorbed phages per ml],

but where number of this density (Titer) of Free Phages is determined indirectly in terms of number of bacteria that are killed.

For example, if you start with 10^8 bacteria/ml, and half are killed upon phage exposure, then your phage Killing Titer is 7×10^7 killing particles/ml, where -ln(0.5) = 0.7. Conversely, a Killing Titer of 7×10^7/ml will result in the killing of half of Targeted Bacteria, given sufficient time for complete Adsorption and assuming a starting density of 10^8 bacteria/ml, i.e.,

$$[\text{“fraction”}] = e^{-[\text{Killing Titer}]/[\text{density of viable bacteria present prior to phage adsorption}]},$$

where ‘e’ is the base on the natural logarithm. See Abedon [263] for an online Killing Titer calculator.

Application of Concept of Killing Titers in Phage Therapy

As with Bacterial Half Life, Killing Titer calculations can be useful toward predicting the maximum possible impact of specific phage Titers on bacterial populations, as well as for assessing the effectiveness of phage treatments given achievement of those Titers *In Situ* [199,262,264]. In particular, if the fraction of bacteria being killed predicts a Killing Titer which is less than the actual starting *In Situ* phage Titer, then phages probably are not efficiently reaching or otherwise Bactericidally Infecting Target Bacteria. Alternatively, if calculations suggest that the Killing Titer is greater than expected then either phage suspensions containing more killing virions than standard Titer calculations can account for, i.e., as based on Plaquing, or instead phages are replicating *In Situ* (for the latter, see Active Treatment).

Killing Titer calculations require at a minimum that all applied phages have successfully Adsorbed, yet one cannot simply assume that MOI_{input} will equal MOI_{actual} (see Multiplicity of Infection— MOI_{input}). Therefore, unless densities of Target Bacteria are quite high, then initial *In Situ* phage Titers will tend to have been greater than the total numbers of those phages which ultimately succeed in Adsorbing over the course of a relatively short experiment. Absent phage *In Situ* Population Growth, there therefore is almost always an expectation of less bacteria killing than starting *In Situ* phage Titers would predict. Thus, if the fraction of bacteria killed by phage action alone is *greater* than that predicted based on starting *In Situ* phage Titers – the latter especially as based on previously *In Vitro* determined Killing Titers for a phage Formulated Product – then that would suggest, as noted, that phage Population Growth and some degree of resulting Active Treatment had occurred.

Latent Period

A Latent Period, generally, is the duration especially of a phage Lytic Cycle. The starting point can be either initial phage Adsorption (see Lytic Infection—Purely Lytic

Infection) or, in the case of Lysogenic Cycles and Temperate phages, the starting point instead can be Prophage induction (see Lytic Infection—Induced Lytic Infection). The end point is Lysis. More specifically for a synchronized population, i.e., given synchronized phage Adsorption in the course of One-Step Growth, the working end-point can either be the start of population-wide Lysis (the start of the what is known as the Rise) or instead the average timing of Lysis (the middle of the Rise). Lysis can be measured either colorimetrically or instead via One-Step Growth experiments.

The importance of Latent Period to Phage Therapy is that it generally is preferred, for the sake of Active Treatment, that phages display relatively short Latent Periods *In Situ*, e.g., not substantially longer than one hour. With Passive Treatment, Latent Period also could be relevant, though more for the sake of the timing of lytic removal of Target Bacteria, assuming Lytic Infections, rather than necessarily toward inhibition of the replication of bacterial populations, as bactericidal activity given Passive Treatment by definition may occur with or without subsequent bacterial Lysis. Latent Period is also relevant to the production of phage stocks, with excessively long latent periods potentially resulting in phages which are more difficult to prepare as stocks. For reviews considering Latent Period and its length, see [265-267].

Lawn

Bacterial Lawns consist of dense, turbid, approximately two-dimensional cultures of bacteria in association with solid or semi-solid media. Bacterial Lawns are utilized in phage biology for visualizing the impact of localized phage Population Growth in the laboratory (Plaque assay) or instead visualization of zones of inhibition of bacterial growth (Spot/Spotting—High-PFU Spotting). Lawns for Plaquing are initiated from cultures of indicator bacteria and may be generated via either pouring or instead via spreading, though pouring is more common in phage work (see Plaque/Plaquing).

Lysate

A Lysate is the product of culture-wide, phage-induced Lysis of a bacterial population (Culture Lysis). During phage stock preparation, the Lysate approximates this initial product, and if not purified to a substantial degree then may be referred to as a Crude Lysate. Crude Lysates, and therefore to various degrees Lysates as well, generally contain a combination of (i) phage particles, (ii) potentially contaminating phage particles (i.e., induced Temperate phages), (iii) bacterial debris, (iv) phage-resistant intact bacteria, (v) bacterial metabolic waste products, and (vi) remaining components of the original culture medium. Living bacteria can be removed via disinfection, filtration, or centrifugation, thereby making a Lysate less Crude. Phages in Lysates

however have *not* been actively separated out of the medium such as via precipitation, chromatographically, via gradient centrifugation, or by fine filtration, with the latter meaning the filtering out of Phage Particles from Lysates versus filtering out larger particles such as bacteria.

Depending on the route of phage administration, or indeed what specifically is being treated (e.g., agricultural fields), then the presence of these other, non-Phage Particle materials may or may not be problematic. For more invasive administration, particularly not topical application nor *per os*, then Lysates generally must be purified into Formulated Products from which potentially harmful, non-Phage Particle ingredients have been removed. Lysate thus is a more general term for something which starts out as a Crude Lysate and which then may be purified via the removal of various components (e.g., bacteria, bacterial debris, or for Gram-negative bacteria, endotoxin) while still remaining a lysate, or instead phages may be mostly removed from the original lysate, resulting in a more purified, non-lysate Formulated Products.

Lysin

Lysin is short for Endolysin.

Lysis

Lysis is a mechanism of Phage Virion Release that results in both destruction of the host bacterium and termination of the phage infection. Lysis for most phages is associated with phage Endolysin release to cell walls from within phage-infected bacteria [268-270] and therefore can be described more formally as a lysis from within. In addition is Lysis from Without, which is more unusual or more artificial than lysis from within. While Lysis from Without also results in the Lysis of bacterial cells, this Lysis does not follow a normal phage Latent Period.

In addition to releasing virions, as well as initiating the solubilization of bacteria and thus solubilizing potentially bacteria-derived toxins, lysis at least in principle may make underlying cells within bacterial biofilms more available to phages (Active Penetration). This is available particularly to those phages released from adjacent lysing bacteria given a Productive Infection (i.e., Auto Dosing), but also is potentially available to phages which are subsequently supplied in the course of extrinsic-to-the-biofilm dosing. In both cases, as noted, such biofilm-associated Lysis would serve as a basis of Active Penetration.

Lysis from Without

Lysis from Without is a mechanism of phage-induced bacterial Lysis that is not dependent upon phage gene expression in association with affected bacteria [271]. Two distinct phenomena have been assigned the moniker of Lysis from Without. Classically this is a Lysis that is associated with high-multiplicity Adsorption of Target Bacteria by T-even-type phages, such as phage T4 (see Multiplicity of Infection and Multiplicity of Adsorption). This Lysis specifically is associated with the gene product 5 of phage T4. This is a virion-associated peptidoglycan-degrading enzyme involved in virion tail tube penetration and then DNA translocation across the Adsorbed host envelope [97,202]. More recently, Lysis from Without has come to be used to describe the consequence of exposing susceptible bacteria to purified Endolysin, that is, Lysis from Without is the antibacterial mechanism of these Enzybiotics. Both usages should be viewed as legitimate.

The Problem with 'Lysis from Without'

It is my opinion that the concept of Lysis from Without in the classical, that is, non-Enzybiotic sense, is overused in the Phage Therapy literature. This is my reasoning: First, suggestions that Lysis from Without has occurred often are based on no evidence except that many phages may have been present. Second, the same phages which display Lysis from Without also display a resistance to Lysis from Without [97], thus making Lysis from Without less likely even if many phages are present, so long as Adsorbed bacteria are metabolizing. Third, not all phage types display Lysis from Without, and indeed so far as we know only a minority of phage types do. Fourth, it is important to keep in mind that phages display Single-Hit Killing Kinetics, and therefore phage-Adsorbed bacteria will tend to be just as killed with or without additional phage Adsorptions and with our without Lysis from Without. Fifth, successful eradication of bacterial populations in fact will tend to require relatively high Multiplicities of Infection (MOI_{actual}) and this is true whether or not Lysis from Without is involved, with this dependence due to phage Adsorptions to bacteria being Poissonally Distributed. Related to the previous point, there simply is no justification for equating Lysis from Without with Passive Treatment even though both by definition, the latter similarly for Poissonal reasons, will require relatively high ratios of Adsorbing phages to Targeted Bacteria.

Care thus should be taken before invoking Lysis from Without in the classical sense as a relevant mechanism during Phage Therapy experiments. Claims of Lysis from Without specifically, and minimally, should be associated with actual demonstrations of Lysis from Without by the phages involved, or at least that Target Bacteria can be Lysed prematurely *In Vitro* – without associated Phage Particle production – given exposure to large numbers of Phage Particles [97].

Lysogenic Conversion

Lysogenic Conversion describes changes to the phenotypic properties of bacteria that can result from the acquisition by bacteria of a Prophage, i.e., this is *conversion* of a bacterium's phenotype upon becoming a *lysogen* [272,273]. The potential for Lysogenic Conversion is one argument against the use of Temperate phages as phage therapeutic agents, and of particular concern is the expression of phage-carried virulence factor genes [274,275]. To a degree, though, this latter issue can be avoided by screening either bioinformatically or phenotypically for the presence of converting genes [276]. Immunity, that is, Homoimmunity, a.k.a., Superinfection Immunity, by contrast is not necessarily described as a product of Lysogenic Conversion, as this is a consequence of lysogenization itself rather than due to expression of additional Prophage-encoded genes [273]. For an essays on lysogenic conversion from a bacterial perspective, see [277,278].

Phage Morons, and Transduction

Associated with the concept of Lysogenic Conversion also is that of phage morons, along with phage-mediated Transduction more generally. Morons are extra or 'more' DNA that is carried within phage genomes, and at least in part this more DNA is associated with effecting Lysogenic Conversion [279]. Transduction here is discussed separately and represents an umbrella term for all phage-mediated movement of especially other-than-strictly phage DNA between bacteria.

Lysogenic

Lysogenic refers to a bacterium which carries a Prophage (a Lysogenic bacterium, a.k.a., a lysogen), or instead refers to a Lysogenic Cycle, which is a phage property. The construct, 'Lysogenic phage', is often used as well, but this is not correct. Use, instead, 'Temperate phage'. Note also that chronically Released phages which are capable of displaying latent cycles, such as phage CTXphi of *Vibrio cholerae*, historically would not be described as Lysogenic, even though they produce Prophages, and this is because these phages do not effect Lysis in the course of Productive Infections. 'Lysogenic', that is, historically would refer to the ability of seeded bacterial lysogens to Lyse bacterial cultures that consist of different bacterial strains.

The concept of lysogeny actually has relatively little bearing on Phage Therapy except to the extent that Temperate phages are actively avoided as treatment phages—Professionally Lytic or at least Strictly Lytic phages instead tend to be preferred as therapeutic phages. In addition, Lysogenic bacteria may be avoided as Propagation Hosts given the potential for these bacteria to produce Temperate phages in the course

of culturing, which will then contaminate subsequently produced Lysates. It is possible, however, to determine both whether Propagation Hosts spontaneously Release these phages and/or whether phage stocks produced using these hosts have been contaminated with induced Temperate phages (see Lytic Infection—Induced Lytic Infection).

Lysogenic Cycle

During Lysogenic Cycles, phages exist as Prophages residing within bacterial lysogens. A phage which is capable of entering into a Lysogenic Cycle is described as Temperate. Contrast Lysogenic Cycle with productive cycle or Productive Infection. Especially for Phage Therapy, contrast Lysogenic Cycle also with Lytic Cycle. Note that Lysogenic Cycles transition to Productive Infections, such as Lytic Cycles, via the process of Prophage induction (see Lytic Infection—Induced Lytic Infection).

Lytic

Lytic refers in various ways to the Release of virions from phage-infected bacteria via Lysis. This is either as the property of a phage or instead as a property of a phage Productive Infection. See Lytic Cycle and Lytic Infection for the latter. As descriptions of the property of phages, see instead Lytic Phage, Professionally Lytic, and Strictly Lytic, with the latter also often described as Obligately Lytic as well as exclusively lytic. So far as is understood, the vast majority of phages are Lytic Phages.

Consistent with there existing a distinction between phage properties and phage-infection properties, note that most Temperate phages are also Lytic Phages (a phage property), but Lysogenic Cycles by definition are not Lytic (a phage-infection property). Thus, the phrase “Lytic or Lysogenic” can be legitimately used to compare Lytic Cycles with Lysogenic Cycles, while neither the phrase “Lytic or Lysogenic” nor “Lytic or Temperate” should be used to compare among phage types. Indeed, the term ‘Lysogenic’ itself literally means ‘Lysis generating’, i.e., essentially Lytic [280].

Lytic Cycle

A Lytic Cycle is a phage life cycle that begins either with virion Adsorption to a bacterium or instead with the induction of a Prophage, and which ends with phage-induced Lysis of the infected bacterium (see equivalently, Lytic Infection). More generally, Lytic Cycles are a form of phage productive cycle (see Productive Infection), that is, where phage virions are both produced and released as Free Phages (called

Release), in this case released via the process of phage-induced bacterial Lysis. Contrast Lytic Cycle therefore not only with Lysogenic Cycle but also with chronic infection, the latter such as seen with filamentous phages (family *Inoviridae*), e.g., phage M13.

For Phage Therapy, Lytic Cycles – due to a combination of bactericidal activity (Bactericidal Infection) and production of new Phage Particles (Productive Infection) – are preferred over Lysogenic Cycles. This is one reason that Strictly Lytic phages, which by definition cannot display Lysogenic Cycles, are preferred over Temperate phages for Phage Therapy (but see as well Lysogenic Conversion as well as Immunity and Transduction as arguments against the use of Temperate phages for Phage Therapy). Most Temperate phages nevertheless display Lytic Cycles, and all tailed phages (order *Caudovirales*) display Lytic Cycles for their Productive cycles. Consequently, most phages in fact display Lytic Cycles.

Lytic Infection

A Lytic Infection is a phage Productive Infection – rather than, e.g., an Abortive Infection – and specifically a Productive Infection which ends with phage-induced bacterial Lysis. As such, a Lytic Infection is synonymous with a Lytic Cycle. I would like to suggest, however, that we might at least conceptually differentiate Lytic Infections into what may be termed 'Purely Lytic Infections' versus 'Induced Lytic Infections'. In any case, all Lytic Phages display Lytic Infections, whether these are Purely Lytic or, for Temperate phages, also Induced Lytic. Note that Lytic Infections, regardless of type, are always both Bactericidal and Productive Infections.

Lytic Infection—Purely Lytic Infection

To the best of my knowledge there is no agreed upon term which unambiguously describes a Lytic Infection which begins with phage Adsorption, versus beginning with Prophage induction. Perhaps one could describe such infections as 'Purely Lytic'. This is rather than 'Strictly Lytic' or 'Obligately Lytic', which instead are terms which are used to describe a type of phage [280]. Note, though, that with Strictly Lytic phages all Productive Infections nevertheless are Purely Lytic. Indeed, for many or most Temperate phages it is thought that many or most Productive Infections *also* are Purely Lytic, that is, rather than most Temperate phage Adsorptions resulting in Lysogenic Cycles or most Temperate phage Productive Infections instead resulting in chronic virion Release.

Lytic Infection—Induced Lytic Infection

Contrasting 'Purely Lytic' would be 'Induced Lytic', that is, Lytic Infections which follow Lysogenic Cycles, thus commencing with Prophage induction. With Temperate

phages there nevertheless are three possible successful infection outcomes following virion Adsorption: (1) Purely Lytic Infection, (2) one or more Induced Lytic Infection following a Lysogenic Cycle, or (3) one or more ongoing Lysogenic Cycles (with more than one Lysogenic Cycle per Adsorption stemming from lysogens, through binary fission, giving rise to multiple lysogen progeny).

For Phage Therapy it is Purely Lytic Infections by Strictly Lytic phages which are preferred. This therefore is rather than Induced Lytic Infections as Strictly Lytic phages by definition cannot display Lysogenic Cycles. It also rather than ongoing Lysogenic Cycles or chronic Productive Infections.

Lytic Phage

Lytic Phages Release their Virion Particles, given Productive Infections, via a process of phage-induced bacterial Lysis. Note that all tailed phages, i.e., phages of virus order *Caudovirales*, are Lytic Phages, and indeed all non-chronically infecting phages, that is, other than phage families *Inoviridae* and *Plasmaviridae*, are Lytic Phages. The term Lytic Phage consequently is *not* a very useful one with regard to Phage Therapy, i.e., it is quite rare for non-Lytic Phages to be used as antibacterial agents.

The utility of the term Lytic Phage has also been hampered by an apparent tendency to equate the concept of Lytic Phage with that of non-Temperate phage. This, however, is a false equivalence. Most Temperate phages, that is, are also Lytic Phages [280], e.g., phage λ. The proper terms for phages which are both lytic and not Temperate instead are Strictly Lytic, Obligately Lytic, Professionally Lytic, or, though I prefer to not encourage its usage, Virulent. This latter term in particular can be associated with additional phage-related concepts besides not Temperate (i.e., see Virulent).

Metabolism (pharmacokinetics)

Metabolism, from a pharmacokinetics perspective, refers to changes in the chemical composition of a drug rather than chemical changes to the body as induced by a drug. For the pharmacokinetics of Phage Therapy, I prefer a broad interpretation of chemical changes to include not just chemical reactions but changes in weak chemical interactions as well. Thus, for phages, pharmacokinetic Metabolism can include changes in virion conformation as well as the binding of immune system molecules to phages, plus all of the changes to phages, including in terms of their gene expression, which are associated with their infection of bacteria.

We can differentiate the impacts of Metabolism into those that are positive, in the sense of increasing concentrations of active drug in the body especially within the vicinity of drug targets, versus those that are negative in that they serve to reduce active-drug concentrations. Phage Adsorption and subsequent phage infection thus tends to result, at least ideally, in phage 'activation' and thereby in positive effects. This in particular is toward Bactericidal Infection where a phage virion is chemically activated into a bacteria-killing infection and/or Productive Infection where a phage virion also is chemically 'activated' into generating more phage virions. Phage interaction with immune systems, on the other hand, can result in both virion sequestration, as due to especially weak chemical interactions with immune system molecules and cells, and virion degradation, e.g., as associated with the breaking of covalent bonds. In either case, the result essentially is phage inactivation, with Metabolism in these cases thereby having negative impacts on phage concentrations *In Situ* [27,87].

Minimum Bactericidal Concentration

See Clearance Threshold.

Minimum Inundatory Dose

Minimum Inundatory Dose refers to the number of Free Phages which must be present in an environment such that the rate of phage Adsorption to Target Bacteria – a function of the product of Free Phage densities and the phage Adsorption Rate Constant – equals the rate at which new bacteria are formed in the course of bacterial replication. If more phages are present, that is, if *In Situ* phage Titers exceed the Inundation Threshold, then bacterial densities will decline over time (see Clearance Threshold), whereas if the number of phages present is fewer than the Inundation Threshold then bacterial densities should increase over time. In all cases, note that we are holding *In Situ* phage Titers constant, that is, we are ignoring the potential for phages to replicate to higher Titers even should bacterial densities exceed what is known as the (phage) Proliferation Threshold, or instead decline to lower Titers. See Payne *et al.*, [82] Payne and Jansen [83] for the mathematical derivation of the Inundation Threshold.

Mixed Passive/Active Therapy

Mixed Passive/Active Therapy is Passive Treatment which nevertheless is aided in its efficacy via Auto Dosing [84]. That is, bacteria are reduced in numbers substantially via Primary Infections (Primary Infection in an Epidemiological Sense) but especially with more rapid and perhaps more complete bacterial eradication accomplished as a

consequence of subsequent *In Situ* increases in phage Titers as due to phage Productive Infections. The result is some degree of Secondary Infection (—Epidemiological Sense) rather than with bacterial killing solely being a consequence of Primary Infections (again, also in an Epidemiological Sense).

Mixed Passive/Active Therapy represents phage therapy taking advantage of the potential for phages to replicate in association with Target Bacteria (i.e., as seen with Active Treatment) while not simultaneously requiring that phages on their own accord increase in numbers *In Situ* to Inundative Densities (i.e., as is required with Active Treatment, but not for Passive Treatment). I have suggested elsewhere that Mixed Passive/Active Therapy, perhaps particularly in combination with multiple phage dosing, may be viewed as what in many instances could represent an ideal strategy for phage therapy [87]: the supplying of large numbers (see Inundative Density) of what nevertheless are still replication competent phages to Target Bacteria; see also [86].

Monophage (Pure Line Phage)

A Monophage is a phage Formulated Product consisting of only a single phage type, e.g., phage T4 in combination with no other phages, i.e., as a Pure Line Phage [47]. Note that the term 'monoclonal' also has been attached to this concept. Contrast with Polyphage. Technically speaking a Monophage can also be a Monovalent phage, or instead can be a Polyvalent phage, while still being a Monophage. This is because the concepts of Monovalent and Polyvalent are properties of individual phages versus Monophage which is, as noted, a property of a phage Formulated Product.

Monovalent

Contrasting Polyvalent, a Monovalent phage is one possessing a relatively narrow Host Range, particularly a Host Range spanning no more than the strains making up a single bacterial species [47,281-284]. In actuality, however, there likely are no phages whose Host Range spans the entirety of even a single bacterial species, and thus a Monovalent phage would be one whose Host Range spans some fraction of only a single bacterial species. The utility of Monovalent phages to phage therapy is that there is less potential for them to impact non-Target Bacteria. To achieve sufficiently broad spectra of activity for Presumptive Treatment, however, Monovalent phages often will need to be mixed into Cocktails.

Note that the concept of Monovalent is different from that of Monophage. In addition, note that the term Monovalent is relatively commonly associated in phage biology with single-charged cations, i.e., monovalent cations such as Na^+ and K^+ (see

Adsorption Cofactor). Note further the concept of "Monovalent phage preparation" [285], which is defined there (p. 180) as "a phage preparation prepared by use of a particular bacterial species and specifically efficient against the chosen bacterial target."

Multiphage

See Polyphage.

Multiplicity of Adsorption (MOA)

Multiplicity of Adsorption (MOA) is equivalent to Multiplicity of Infection (MOI), though only when the concept of Multiplicity of Infection is used as equivalent to MOI_{actual} [35,286], that is, as the ratio of numbers of *Adsorbed* virions to numbers of Target Bacteria. MOA as a term is not commonly used by phage biologists, however. It nevertheless is included here because it helps to clarify the concept of Multiplicity of Infection as MOI_{actual}.

Multiplicity of infection (MOI)

Multiplicity in phage biology refers to the ratio of especially Phage Particles to Target Bacteria [35]. There are two interpretations to the concept of Multiplicity of Infection (MOI). These can be described as MOI_{actual} versus MOI_{input}.

Multiplicity of Infection—MOI_{actual}

MOI in classical terms is the ratio of Adsorbed phages to Target Bacteria. From Benzer *et al.* [44], p. 144, "Since Adsorption of phages is never 100%, the actual multiplicity has to be determined for each experiment…" and from Adams [47], p. 441: "Multiplicity of infection: Ratio of Adsorbed phage particles to bacteria in a culture." That definition, as noted, has come to be seen as only one interpretation of MOI, so-called MOI_{actual} [257]. It is important to appreciate, though, that MOI_{actual} *is* Multiplicity of Infection, both in terms of usefulness and as the concept was originally defined ("infection" here can be interpreted as equivalent to "Adsorption" or "Attachment", i.e., see Multiplicity of Adsorption). Multiplicity of Infection as MOI_{actual} is important especially for describing Poisson Distributions of Adsorbed phages over phage-Targeted Bacteria, and also (equivalently) for determining phage Killing Titers. See Abedon [287] for an online Multiplicity of Infection as MOI_{actual} calculator.

As the following section on MOI_{input} should make clear, ideally all references to Multiplicity of Infection would be referring to MOI_{actual} unless otherwise indicated. Beware, however, that in a large fraction of publications it appears to be MOI_{input} which is used instead, though this usage is not often explicitly indicated. Note that MOI_{actual} also has been described as an effective Multiplicity of Infection [192].

Multiplicity of Infection—MOI_{input}

The alternative interpretation of Multiplicity of Infection is as MOI_{input}, which is the ratio of numbers phages *added* to a bacterial culture to numbers of Target Bacteria in that culture, and this is rather than the number of phages which necessarily have Adsorbed [257]. This definition of MOI represents a shortcut which can be taken when rapidly Adsorbing virions are added to high densities of bacteria, e.g., $>10^7$ bacteria/ml, since then fast Adsorption by most added phages is expected, resulting in MOI_{input} coming to approximate MOI_{actual} (where MOI_{actual}, as noted above, should represent the goal of MOI descriptions). This MOI_{input} approximation, however, (i) can be imprecise, (ii) generally should be experimentally verified before being relied upon, and (iii) particularly should be verified if the Adsorption characteristics of a given phage under a given set of conditions or to a given Target Bacterium are not otherwise known. Implicit claims that MOI_{input} might approximate MOI_{actual} in other words can in many cases represent simply a guess. Furthermore, given low bacterial concentrations, i.e., roughly $<10^7$ bacterial/ml, then MOI_{input} will almost always be expected to fail to approximate MOI_{actual}, resulting in Multiplicity of Infection (as MOI_{input}) being a somewhat irrelevant measure toward appreciating the dynamics of phage interactions with bacteria, such as during Phage Therapy.

Many studies also describe dosing during Phage Therapy experiments solely in terms of MOI_{input}, while often also leaving bacterial densities poorly indicated. This practice makes it difficult or even impossible to ascertain what numbers of phages in fact were added to Target Bacteria during dosing, which in turn can result in published experiments being largely not replicable, and even uninterpretable except broadly. Furthermore, it is unlikely that Phage Therapy in actual practice will tend to be dosed in terms of a given MOI_{input}, i.e., versus instead in terms of phage Titers and volumes. As a consequence of these issues, use of MOI_{input} should be strongly discouraged when reporting on Phage Therapy unless justification for its use can be provided. MOI_{actual}, by contrast and as noted, is both legitimate and useful as a measure during experiments, though it too should not be used as a sole description of dosed phage numbers [36].

Numerical Refuge

The concept of a Numerical Refuge describes circumstances where insufficient bacterial densities are present to support phage Population Growth, especially growth to Inundative Densities. From Chao *et al.* [288], p. 375: "When the phage and bacteria are sparse, the prey population [i.e., the bacteria] can increase with near impunity but support little growth of the predator population [i.e., the phage]. However, when the density of this primary consumer population is great [again, the bacteria], the opposite is true. Now the phage thrive and, if they were not originally plentiful, they soon become so. This will halt the growth of the bacterial population."

Related Concepts

In pertaining to Phage Therapy, a numerical refuge refers to Target Bacteria being present at insufficiently high densities to support successful Active Treatment. Bacterial densities at a Proliferation Threshold, which is that bacterial concentration required to support the ongoing persistence of Strictly Lytic phages, also are insufficient to support Active Treatment. Nevertheless there is no obvious equivalency between bacterial densities which would define a Numerical Refuge and those which would define a Proliferation Threshold: Are Numerical Refuge densities always lower than Proliferation Thresholds? Lower than or equal to? Possibly even slightly greater than? Nevertheless, by definition in neither case are bacterial densities sufficiently high to support phage Population Growth to Inundative Densities. Numerical Refuges also may be defined as essentially non-winner bacterial densities (see Active Treatment for discussion).

Obligately Lytic

Obligately Lytic describes phages which both Release virions Lytically and are not Temperate, i.e., which can infect successfully only via Lytic Cycles. Equivalently, see Strictly Lytic. To a first approximation, Obligately/Strictly Lytic phages are preferred for phage therapy. See also Professionally Lytic.

One-Step Growth

One-Step Growth experiments are a means of simultaneously determining the Burst Size and Latent Period of a phage as it infects a specific bacterial host. This involves synchronizing the Adsorption (i.e., Attachment) of phages at relatively low Multiplicities of Infection but nevertheless promoting relatively complete Adsorption of the phage population. It also involves subjecting cultures to post-Adsorption diluting to prevent Secondary Infection—Biomedical Sense, i.e., the initiation of new infections. Resulting phage infections are then followed in terms of infective centers, i.e., Plaque-forming units consisting of either Free Phages or phage-infected bacteria, through

Culture Lysis and associated Rise [111]. One-Step Growth is also known as Single-Step Growth. For further discussion of One-Step Growth as well as experimental protocols, see {Ellis, 1992 2797 /id;Carlson, 1994 1403 /id;Carlson, 2005 11675 /id;Hyman, 2009 11222 /id;Kropinski, 2018 38493 /id;Abedon, 2025 45297 /id}.

Lysis Profiles and Multi-Step Growth

Note that technically One-Step Growth experiments should *not* be done at higher phage Multiplicities of Infection (MOI), i.e., MOIs approaching or exceeding 1, since the intention is to determine the properties especially of singly phage-infected bacteria (see Poisson Distribution). As a consequence, lysis profile experiments where one follows phage infections in terms of changes in culture turbidity over time – resulting Culture Lysis here is associated with a drop in turbidity – are technically not One-Step Growth experiments. This is even if they are initiated with simultaneous phage Adsorption of a majority of the bacteria present and consequently result in a single drop in culture turibidity. There certainly can be equivalence between lysis profiles and One-Step Growth experiments, however, in terms of the measure of resulting phage Latent Periods. Experiments which follow phage Population Growth through more than one round of Adsorption, infection, and then Lysis are also, without question, not examples of *One-Step* Growth as multiple 'steps' of Lysis and Adsorption in that case are explicitly allowed to occur.

Passive Treatment (Passive Therapy)

Passive Treatment, as equivalent to Inundation Therapy, is Phage Therapy that can be successfully accomplished in the absence of *In Situ* phage Population Growth, i.e., without Auto Dosing. Such success requires an achievement, via the action of extrinsically supplied phages alone, of phage Titers *In Situ* which are equal to or greater than what can be described as Inundative Densities. Contrast Passive Treatment with Active Treatment. See also Purely Passive Treatment and Mixed Passive/Active Treatment.

Penetration

Penetration is a term that can be used to describe, in combination, the pharmacokinetic concepts of Absorption and Distribution as well as the movement of phages into bacterial biofilms. For the latter, as in the course of effecting Active Penetration [59], phage Penetration likely serves as an important parameter in determining phage potential to display Anti-Biofilm Activity [289]. Penetration thus is a process of Phage Particle translocation from a point of dosing to a point of Encounter

with one or more Target Bacteria, and this especially is where dosing and Encounter take place (i) within pharmacologically different 'compartments' within a body, (ii) in association with a biofilm, or (iii) or otherwise in different locations with regards to a larger environment.

Performance

Phage Performance describes a spectrum of activity regarding a phage's ability to negatively impact Target Bacteria and/or as positively impacts phage Population Growth. In terms of phage infections, phage Performance can range from (i) inability to adsorb at all to (ii) failure to achieve Bactericidal Infections (e.g., restricted infections) to (iii) achieving Bactericidal Infections (e.g., Abortive Infections) to (iv) resulting in Productive Infections to (v) displaying highly Productive Infections, i.e., especially large Burst Sizes for the latter, but also reasonably short phage Latent Periods. Thus, high Infection Vigor would be equivalent to high phage *infection* Performance.

Purely Passive Treatment requires only Bactericidal Infections so therefore requires lower phage Infection Performance than Active Treatments. That is, Active Treatments require Productive Infections or even highly Productive Infections rather than just Bactericidal Infections. An ability of phages to overcome mechanisms of bacterial resistance to phages, e.g., such as Abortive infections, can contribute to improved phage infection Performance, i.e., the transition from possibility (iii) to possibility (iv) in the previous paragraph. A phage's Performance for Phage Therapy can also be functions of phage Adsorption Rates to Target Bacteria, as well as phage Host Range, i.e., with faster Adsorption Rates, greater Adsorption Affinity, or broader phage Adsorptive Host Ranges potentially indicating greater phage anti-bacterial Performance.

Generally greater phage Performance is desirable during Phage Therapy, i.e., from Rohde *et al.* [144], p. 3, phages should "show important infectious ability, such as a broad Host Range, high efficiency of plating (EOP), high Adsorption Rates, short Latent Periods, large Burst Sizes and a low inclination to select resistance". To a degree, however, it can be possible to compensate for lower phage Performance – particularly regarding lower Burst Sizes or slower rates of Adsorption, but also lower survival ability *In Situ* [290] – by dosing with greater numbers of phages. Note that Phage Performance alternatively may be equated with 'phage treatment performance', which though presumably a function at least in part of Phage Performance as defined here, can be dependent as well on additional factors such as phage delivery strategies.

Permissive

Permissive refers to bacterial hosts and/or environmental conditions which are able to support phage Population Growth. This is particularly, though not exclusively, toward Plaque formation, with Permissive hosts or conditions supporting relatively high Efficiencies of Plating.

Phage Bank

Phage Banks, sometimes also referred to as phage libraries or phage repositories, are collections of previously isolated and characterized phages [291], ones which can then be individually tested against to-be-treated bacterial etiologies. i.e., Target Bacteria. This contrasts with the use of off-the-shelf phage products (Prêt-à-Porter) as well as contrasting with the isolation of a phage against an etiology obtained from a specific patient to be used for treatment specifically of that patient (Autophage). Use of a Phage Bank, however, is not inconsistent with the use of Cocktails since the phages making up a Cocktail can be chosen for treating a specific patient from a Phage Bank. Indeed, the phages making up a Cocktail as derived from a Phage Bank could be targeted toward different etiologies, given treatment of a mixed infection.

Phages from a Phage Bank may be tapped should the phages initially used to treat an infection, including Presumptively, turn out to be insufficiently efficacious. Phage Banks, however, will tend to be less useful for prophylactic phage use, unless that strain of Target Bacterium which is being controlled prophylactically is known with some precision beforehand. For further discussion of Phage Banks, see Pirnay *et al.* [130], Chan and Abedon [155], Chan *et al.* [156], and Pelfrene *et al.* [292].

Phage Library

Note that the alternative and more common usage of the term ‘phage library’ is to describe single preparations of multiple different recombinant phages, e.g., as cloned into a phage lambda vector or for use in phage display. This is rather than a collection of multiple pure line phage isolates (Monophages) present in multiple pure stocks, i.e., as equivalent to a Phage Bank. This non-Phage Bank meaning of phage library is potentially relevant to antibacterial phage therapy to the extent that a phage library consists, for example, of multiple random iterations of a phage gene such as involved in Target Bacterium recognition, and toward modification of phage Host Range as may be generated within a single phage stock toward subsequent selection. Thus, for the sake of avoidance of ambiguity, it is best to not equate Phage Bank with phage library despite the obvious equivalence of ‘bank’ and ‘library’ as repositories of well segregated entities (e.g., accounts versus books), with segregation in Phage Banks between separate phage

stocks versus segregation in phage libraries generally between separate Phage Particles found within the same stock.

Phage Escape Mutant

Phage Escape Mutants are phages which have overcome bacterial Resistance mechanisms, such as Abortive Infection systems [67,293-298], via mutation. Note, however, that the concept of 'escape mutant' is used much more broadly than just in terms of phage mutations. In addition, the term Phage Escape Mutant has also been used equivalently to Bacteriophage Insensitive Mutant (BIM) [129], though for the sake of minimizing ambiguity, this equivalent usage should be avoided.

Phage-Mediated Biocontrol of Bacteria

See Biocontrol.

Phage Particle

Phage Particles generally are the active ingredients in phage Formulated Products. The term is equivalent to virion or Virus Particle. If found outside of a bacterium, then a Phage Particle also can be referred to as a Free Phage.

Phage Tail-Like Bacteriocin

See High Molecular Weight Bacteriocin.

Phage Therapy

Phage Therapy is the use of bacteriophage Virus Particles to combat bacteria, especially within medical or veterinary contexts, i.e., as in the antibacterial treatment of individual, diseased patients or animals using phages. See, equivalently, Bacteriophage Therapy. Phage Therapy also can be viewed as a form of Biocontrol, i.e., as mediated using phages as the Biological Control agent. A discussion of what is Phage Therapy versus what instead may be described more generally as phage-mediated bacterial Biocontrol can be found in Abedon [43]. Here are some Phage Therapy 'best practices' articles [35-37,299].

Phages

Phages is the plural of phage. So long as a publisher will allow it, then 'Phages' may be employed when considering more than one type of phage, e.g., 'Phages T4 and T7', and also when describing a collection of 'Phages' of the same type, i.e., '20 ml of 10^8 phages/ml were applied to the bacterial infection'. As this usage has not been consistent in the phage (singular) literature, my tendency is to substitute an alternative term possessing less ambiguity as a check, e.g., "the horses Frankie and Diamond where set loose into the paddock" or "two horses are a lot of horses to feed". Less obviously but still surmountable, note that it is a 'herd of wild horses' (as a stand-in for, e.g., a 'stock of T4 phages'), rather than a 'herd of wild horse', which may be set loose into a field, that is, as the stock of phages rather than stock of phage may be added to a bacterial culture. An historical and clarifying essay on this usage is provided by Ackermann [300]. Nevertheless, it is clear that phage or bacteriophage as plurals can be found throughout the phage and Phage Therapy literatures.

Phage Steering

Term invented [301] to describe the use of phages to deliberately 'steer' bacterial resistance evolution toward less virulent bacterial genotypes, such as by using phages that target in their *Adsorption* surface-located bacterial virulence factors.

Plaque/Plaquing

A Plaque is a region of reduction in bacterial numbers which is associated with localized phage Population Growth within spatially structured environments. Such regions are commonly seen upon plating phages together with indicator bacteria either on or, more commonly, within solidified agar in Petri dishes. Plaques are important for enumerating phages as well as toward first-approximation characterization of phages, including in terms of Host Range. Plaque-forming units (PFUs) are entities, such as Free Phages, which are capable of generating a single plaque upon plating.

Plaquing-based or plaque-utilizing assays include those of Efficiency of Plating, Efficiency of Center of Infection, and also One-Step Growth experiments. Contrast, however, Spotting using high phage numbers (Spot/Spotting—High-PFU Spotting) which generally will result from the lytic action of large numbers of PFUs rather than that of a single PFU, as ideally is the case for a single plaque. For more on Plaques, their formation, and protocols, see [56,151,195,198,199,261,302-306]. For a chapter on statistical aspects of Titering using Plaque assays, see [307].

Poisson Distribution

A Poisson Distribution is a statistical concept used to describe the likelihood of individual, discrete events occurring, given some *average* likelihood of such events occurring [31-33,308-310]. In terms of phages, this can be seen as the likelihood of a specific number of phages Adsorbing to individual bacteria given some average number of adsorptions per phage-susceptible bacterium. The latter quantity is Multiplicity of Infection or, more precisely, MOI_{actual}. For Phage Therapy, the most useful of these likelihoods is that of no phage Adsorptions, i.e., the proportion of phage-exposed bacteria where the number of resulting phage adsorptions is equal to zero, as this is the fraction of bacteria which will have escaped phage infection given Adsorption of a certain number of phages to a certain number of bacteria. This no-Adsorption value is equal simply to e^{-M} where M is MOI_{actual} and e is the base of the natural logarithm.

With MOI_{actual} = 1, for example, then the fraction of bacteria which are expected to escape phage Adsorption is 37%. In addition, for MOI_{actual} = 1, the fraction which are expected to have been Adsorbed by only a single phage also happens to be 37%. The number of 'missing' phage adsorptions, that is, other than those which have been singly Adsorbed, instead are those found multiply Adsorbed to individual bacteria. For MOI_{actual} = 1, these multiply Adsorbing-to-the-same-bacterium phages represent 63% (i.e., 100 - 37) of the total number of Adsorbed phages, while the fraction of bacteria which are multiply phage Adsorbed are 26% (= 100 - 37 - 37) of the total number of Target Bacteria. Thus, 59% of *phage-Adsorbed* bacteria in this example are singly Adsorbed (37/(37+26)) while the remaining 41% of phage-Adsorbed bacteria are multiply Adsorbed. A Poisson Distribution frequencies calculator can be found online at [311].

Inundation

Because phage Adsorptions are distributed Poissonally rather than evenly over Targeted Bacteria, it is necessary for many more than one phage Adsorption per individual Targeted Bacterium to occur to result in substantial bacterial eradication, i.e., as illustrated in the previous paragraph. With an MOI_{actual} of 10, then the fraction of bacteria which are expected to escape phage Adsorption is equal to $e^{-10} = 4.5 \times 10^{-5}$, or roughly one in 20,000. If lower bacterial survival than one in 20,000 is required, assuming all bacteria are equivalently phage susceptible, then an MOI_{actual} of greater than 10 would be required. Thus, to achieve substantial bacterial eradication then a fairly high MOI_{actual} is required, and this is the case independent of any potential for treatment phages to induce a Lysis from Without. See especially Killing Titer calculations for application of the Poisson Distribution to Phage Therapy, and also the various concepts of Inundation of bacteria.

Polyphage (Multiphage)

A Polyphage is a mixture of multiple phage types, as equivalent to a phage Cocktail [312-314]. Alternatively, some instead use the term 'Multiphage' [315-317]. Thus, phage Cocktail, Polyphage, and Multiphage are synonymous.

Polyphage also is used to describe individual virions which contain more than one genome [318], e.g., [319-322]. Polyphage has been used as well seemingly to mean Polyvalent, with Monophage thereby used equivalently to Monovalent [323]. It should be noted however – for the sake of preventing ambiguity – that this latter sense, though it is not consistent with usage elsewhere in the phage literature and therefore should be avoided, nevertheless *is* consistent with the more general, non-phage definition of Polyphage, as an equivalent to the concept of omnivore.

Polytherapy

See Combination Therapy.

Polyvalent

The term Polyvalent is a description of a phage's Host Range, one is which is equated in many contexts with a 'broader' Host Range, contrasting Monovalent which would refer instead to a 'narrower' Host Range. More technically, the term Polyvalent should be reserved to describe, at the least, Host Ranges for individual phages which span multiple bacterial species [47] or, alternatively, which span multiple bacterial genera [324-326]. The term Polyvalent also may have been used equivalently to Polyphage, p. 122 [47]: "…the term polyvalent phage was also applied to mixtures of phages prepared for therapeutic use, and it is often difficult to tell in the early literature whether a 'polyvalent phage' was a 'pure line phage' or a mixture of phages." Because of its vagueness as well as diversity of 'definitions' mostly implicitly employed in different publications, the concept of phage Polyvalence, as a term, often is not very useful.

Population Growth

From ecology, Population Growth occurs when a population's 'birth' rate exceed its 'death' rate, thus resulting in net gains in population size. Active Treatment by definition is dependent on phage Population Growth as that occurs *In Situ*, while phage stock generation too requires phage Population Growth, though as occurs *In Vitro*. Note,

however, that Population Growth is not identical to simply the occurrence of replication, or indeed to Auto Dosing, since numbers of individuals within a population must net increase for population growth to occur. This is versus remaining constant, where for phages the latter is seen given host bacterial densities equal to Proliferation Thresholds. It is also versus declining phage population sizes despite ongoing phage replication (which conceptually simply means that deaths exceed births). In addition, for Phage Therapy to be successful, then at a minimum Target Bacterium deaths must exceed Target Bacterium Births.

Presumptive Treatment

Presumptive Treatment refers to the Initiation of medicament dosing prior to full confirmation of laboratory-determined susceptibility of a condition to that treatment. With antibacterial agents this would be initiation of treatment prior to confirmation of Target Bacteria sensitivity *In Vitro*. Presumptive Treatment of bacterial infections saves time, labor, and laboratory fees, but requires prescription of sufficiently broadly acting agents that all or at least most likely etiologies are sensitive.

Because the Host Range of phages tends to be relatively narrow, the potential especially for individual phages to be used presumptively is lower than that for the typically more broadly acting antibiotics. To a degree, however, this issue can be addressed for phages by treating with Cocktails consisting of phages possessing a diversity of Host Ranges. Similar Issues to presumptive phage use are seen with phage use prophylactically. That is, preventing infections by bacteria also can involve targeting etiologies possessing otherwise unknown phage susceptibilities. It is important to note as well that antibiotic resistance as acquired by pathogens also results in lowered potentials for successful Presumptive Treatment using antibiotics. For further discussion of Presumptive Treatment with regard to phage therapy, see Chan and Abedon [155] and Chan *et al.* [156].

Prêt-à-Porter

Prêt-à-Porter literally means ‘ready-to-wear’, or idiomatically, ‘off-the-shelf’ but, as used by Pirnay *et al.* [130] refers to non-customized phage Formulated Products which are designed to be broadly applicable, contrasting Sur-Mesure products. Typically a Prêt-à-Porter phage Formulated Product would be a Cocktail. Not all phage Cocktails are necessarily Prêt-à-Porter, however, as Cocktails can alternatively be developed such as from Phage Banks to act against specific bacterial isolates and/or for use against specific bacterial infections. Nevertheless, phage Cocktails as commercially available Formulated Products represent Prêt-à-Porter phage therapeutics as typically envisaged.

Primary Infection

Primary Infection refers either to the first phage to reach and infect a bacterium (contrast Secondary Infection—Biomedical Sense) or instead the infection of a bacterium by a phage which has been supplied other than by Auto Dosing (contrast Secondary Infection—Epidemiological Sense). With Passive Treatment, all phage infections in principle could be Primary Infections (*sensu* epidemiology) whereas with Active Treatment by definition phage infections cannot all be Primary Infections (again, *sensu* epidemiology). That is, with Active Treatment *In Situ* phage Population Growth is required to achieve Inundative Densities of phages, and the resulting newly formed phages by definition would give rise to Secondary Infections in an Epidemiological Sense rather than give rise to new Primary Infections (also in an Epidemiological Sense).

Primary Infections in a Biomedical Sense, by contrast, are ones which can follow either normal dosing or instead result from Auto Dosing, since they simply are derived from the first phages to reach and infect a given bacterium. These also are the infecting phages which express such things as Immunity or superinfection exclusion (for the latter, see Secondary Infection—Biomedical Sense).

The phages which reach a bacterial population through standard dosing (not Auto Dosing) thus generate Primary Infections in an Epidemiological Sense, whereas the progeny of those phages, products of Auto Dosing, instead produce Secondary Infections, also in an Epidemiological Sense. In considering individual bacteria, however, the first phage to adsorb will produce a Primary Infection and subsequently Adsorbing phages to the same bacterium will represent Secondary Infections (or, at least, secondary adsorptions), with both terms from this latter perspective used in a Biomedical Sense. See Secondary Infection for further discussion.

Productive Infection

A phage Productive Infection is one that gives rise to and releases functional Phage Particles, i.e., a phage infection which produces Free Phages (thus, a 'Free Phage-Productive Infection'). Virion Release can be either via Lysis (Lytic Infection) or instead can occur chronically, the latter, e.g., as seen with phage M13. Productive Infections are a necessary but not sufficient requirement for positive phage Population Growth – growth as virions versus as Lysogens – and therefore for successful Active Treatment. Productive Infections are *not* sufficient for successful Active Treatment because bacterial densities must be present above a Proliferation Threshold for *net* phage Population Growth to occur, and even net phage Population Growth may not be

sufficient for phage populations to reach the Inundative Densities required for successful Active Treatment.

By definition, Productive Infections are *not* required for Purely Passive Treatment as this necessitates only Bactericidal Infections by phages. See, however, Mixed Passive/Active Therapy for which Productive Infections do play a role. The infection Performance required of a Lytic Phage to achieve a Productive Infection, and thus to potentially result in successful Active Treatment, should generally be assumed to be greater than that level of infection Performance required instead to achieve an only Bactericidal Infection, and thereby only Passive Treatment. Infection Vigor similarly is a description of degrees of Productive Infection Performance.

Professionally Lytic

A Professionally Lytic phage is one that is both Strictly Lytic and not closely related, genetically, to a Temperate phage [280]. That is, not all Strictly Lytic phages are *not* recent descendants of Temperate phages but instead may be derived via a mutational knocking out of genes required for lysogeny establishment (see Virulent—Temperate Phage Mutant as Virulent). One utility to not employing for Phage Therapy phages that are closely related to Temperate phages is to minimize recombination events between therapeutic phages and resident Prophages, either *In Situ* or in the course of phage stock preparation. Another utility is a lower potential for a therapeutic phage to encode bacterial virulence factor genes, as by definition Professionally Lytic phages are not closely related to phages that are capable of effecting Lysogenic Conversion.

Proliferation Threshold

A Proliferation Threshold is that bacterial density, such as in colony-forming units per ml, which can support sufficient phage Population Growth to offset rates of Phage Particle inactivation. The idea is that a given Phage Particle can either adsorb to a bacterium and give rise to a Productive Infection or instead become inactivated. The rate of virion Adsorption in part is a function of bacterial density whereas the rate of especially bacterial host-independent virion inactivation is a function of other environmental properties. Thus, for the calculation, Phage Particle per-capita inactivation rates are held constant at some level, as too is the phage Adsorption Rate Constant. The Proliferation Threshold consequently is approximately that bacterial density for which rates of virion Adsorption for an entire Burst Size of phages equals rates of virion inactivation. Thus, $NkB \approx I$, where N is the Proliferation Threshold, k is the

phage Adsorption Rate Constant, *B* is the phage Burst Size, and *I* is the rate of phage inactivation.

At bacterial densities that are higher than the Proliferation Threshold, phage Population Growth should ensue. A concentration of Target Bacteria which is greater than the Proliferation Threshold thus is necessary for successful Active Treatment to occur, though not sufficient. That is, for Active Treatment to be successful then not only must bacterial densities exceed the Proliferation Threshold, but also must be sufficiently high in density to, in addition, support phage Population Growth to Inundative Densities. For additional discussion of Proliferation Thresholds, see [27,82,83,86,153,327,328].

Phage Reproductive Number of One

The Proliferation Threshold also is that bacterial density which would support an R_0 value equal to 1. R_0, from epidemiology, is the number of subsequent infections per initial infection (number Secondary Infections per Primary Infection, both in an Epidemiological Sense). For the phage reproductive number, this is the number of new phage-infected bacteria that each phage-infected bacterium on average gives rise to. An R_0 value of 1 thus is each phage on average succeeding over time only in replacing itself, which is what is sustained given Proliferation Threshold bacterial densities.

Effective Burst Size of One

An equivalent perspective on Proliferation Threshold is that it is that bacterial density which is capable of supporting a phage *Effective* Burst Size of 1, meaning that only one phage per Burst per phage-infected bacterium survives to initiate a new infection (Secondary Infection—Epidemiological Sense). Thus, at the Proliferation Threshold, Effective Burst Size = R_0 = 1. Again, at Proliferation Threshold bacterial densities, each phage on average only succeeds in replacing itself.

Propagation Host

A Propagation Host is a bacterial strain used to generate phage stocks. Ideally for Phage Therapy this bacterium will be relatively non-pathogenic, not otherwise carry Transducible bacterial virulence-factor genes, nor carry either inducible Prophages or even Prophage sequences with which propagating phages can recombine. Ideally as well, there will be a relative ease of propagation and handling of the Propagation Host along with a good potential for it to support the generation of high-Titer stocks of the propagated phage. Indeed, to the extent that a Propagation Host is valuable, then phage choice during Formulated Product development may be biased toward those phages

which are readily propagated on that strain, at least to the extent that such a bias does not greatly limit the ultimate therapeutic potential of those phages which are chosen for further development. Note that the concept of host bacterium is broader than that of Propagation Host, which instead is a specific strain of all possible host bacteria for the propagated phage.

Prophage

A Prophage is a Temperate phage, particularly its genome, as it exists during a Lysogenic Cycle. A bacterium possessing at least one functional Prophage is described as a lysogen (noun), or Lysogenic (adjective). A polylysogen in turn possesses multiple distinct Prophages per bacterium.

Prophages are relevant to Phage Therapy particularly due to their ability to express Immunity against homoimmune phages, which thereby can render Target Bacteria resistant to therapeutic phages. Such immunity should be an issue, however, only if therapeutic phages are Temperate, so therefore should be somewhat less of an issue given use of Strictly Lytic therapeutic phages. In addition, Prophages if present within Propagation Hosts, and induced, can contaminate phage stocks with resulting virions [144].

Pseudolysogeny

The term Pseudolysogeny has different meanings to different authors but generally should be viewed as a consequence of an infecting phage in some manner mimicking a Lysogenic Cycle, but only superficially. I tend to strongly discourage use of the term, however, except when referring to its usage by others. I would also strongly encourage that an explicit definition be provided whenever the term is used since otherwise it is impossible to tell what phenomenon is being considered under this heading. Pseudolysogeny, that is, simply cannot be understood unambiguously as a single concept because historically it has been used to describe multiple phage-associated phenomena. For a list of the numerous definitions that have been attached to the concept of Pseudolysogeny, see Abedon [55]. Note that the term carrier state is also sometimes used synonymously with Pseudolysogeny, and use of that term similarly can be problematic.

Pure Line Phage

See Monophage.

Purely Passive Treatment (Pure Passive Therapy)

Purely Passive Treatment is equivalent to Passive Treatment but emphasizes a lack of contribution to bacteria-killing efficacy by Auto Dosing. This can be viewed as a means of distinguishing this Purely Passive Treatment from Mixed Passive/Active Therapy. When employing phages which are capable of achieving Bactericidal infections but are not able to Productively infect, then Purely Passive Treatment by definition is the only possible route toward efficacious Phage Therapy. Note that Payne and Jansen [84] emphasize the point, of a lack of requirement for phage replication to achieve bacterial eradication given Passive Treatment, by instead using the phrase, p. 319, “pure passive therapy”, though grammatically I tend to prefer the phrasing “Purely Passive Treatment” (or “Therapy”).

Receptor

Receptor, in phage biology, refers especially to molecules found on the surfaces of bacteria to which Phage Particles bind in the course of Adsorption and Attachment. Phage Receptors should not be confused with those molecules that are associated with Phage Particles which bind to these bacterial surface molecules. Which phage Receptors are present on the surfaces of bacterial species and strains play large roles in determining phage Host Range.

Release

Release is the transition of intracellular located phage virions to the extracellular environment. This can occur via either phage-induced bacterial Lysis or instead via non-Lytic mechanisms (chronic release). Release also can occur as a consequence of artificial bacterial Lysis, e.g., as was employed by Doermann [185,186] toward discovery of the phage Eclipse.

Resistance

Resistance describes especially an acquired interference by a bacterium with the actions of an antibacterial agent. Specifically, bacterial sensitivity to an agent is reduced in the laboratory, i.e., *In Vitro*, and to an equivalent extent is reduced *In Situ* as well, and this reduction in sensitivity is associated either with a bacterial mutation or instead occurs via the acquisition of new genetic material by bacteria via horizontal gene transfer. See for example Abortive Infection but also, under Synergy, see the concept of Evolutionary Synergy. Contrast, however, the concept of Resistance with that of

Tolerance. In any case, note that Resistance is a bacterial property rather than a phage or antibiotic property, though phages can evolve to overcome bacterial Resistance. See also Cross Resistance. Contrast with phage Tolerance. Reviews on bacterial Resistance to phages as relevant to Phage Therapy include [144,163,164,282,314,329-335]. For a systematic look at bacterial Resistance to phages, see [69,70,336,337]. Consider also the related concept of phage-bacterial antagonistic coevolution [338,339].

Rise

Rise refers to the increase in phage numbers, particularly as seen upon phage-induced bacterial Lysis during One-Step Growth experiments [340]. Thus it is literally a Rise in phage Titers, i.e., as required *In Situ* for successful Active Treatment. Alternatively, the term Rise has been used to describe the intracellular increase in phage numbers as occurs during Lytic Cycles, thus as equivalent to the virion-maturation or post-eclipse stage of these phage infections. For the sake of reducing ambiguity, however, this latter, newer usage should be discouraged.

Secondary Infection

Secondary Infection can refer either to the infection of bacteria by those Phage Particles which have been generated *In Situ* such as occurs in the course of Active Treatment (an Epidemiological Sense of the concept) or instead can refer to the Adsorption of an already phage-infected bacterium by another phage (a more Biomedical Sense of the concept). Because there is more than one meaning of the term, it would be helpful were authors to specify their intended meaning when it is not otherwise obvious from context. For an essay on these various facets of Secondary Infection including as pertains to Phage Therapy, see Abedon [341].

Secondary Infection—Epidemiological Sense

Secondary infection in an epidemiological sense is the underlying basis of Active Treatment. Here the epidemiology is as occurs within a treated patient, or for Biocontrol within a treated environment, and this is the infection of bacteria by *In Situ* generated phages, that is, as generated in the course of Auto Dosing. Thus, the originally dosed phages give rise to Primary Infections while the phages produced by *In Situ* bacterial infections give rise to Secondary Infections, that is, phage infections of additional bacteria [82,83,342]. The analogy is to the propagation of a parasite through a population of hosts, where the first individual to be infected within the host population supports the Primary Infection, and with subsequent hosts infected by parasite progeny of the Primary Infection, thus supporting Secondary Infections.

Secondary Infection—Biomedical Sense

Secondary Infection in a biomedical sense – meaning an infection which occurs on top of or following an already existing infection – results in the loss of phage killing power. Such losses occur because a bacterium which has been Adsorbed by only a single phage is, ideally, no less dead than a bacterium which has been Adsorbed by multiple phages (see Single-Hit Killing Kinetics). Furthermore, generally a single bacterium should be able to support no more than one phage Burst. See, however, Poisson Distribution for appreciation of why the Adsorption of multiple phages to individual Targeted Bacteria nonetheless is still preferable in the course of Phage Therapy versus Adsorption of bacteria by no more, on average, than only a single Phage Particle.

Related or associated terms, especially in this biomedical sense of the concept of Secondary Infection are superinfection, coinfection, and also secondary Adsorption, plus see also Lysis From Without, as well as the concept of lysis inhibition [54,97,152,343]. Note that the Adsorption of a phage to a bacterial lysogen also can be considered to be a form of Secondary Infection, e.g., as potentially giving rise to Superinfection Immunity, with in this case infection being secondary to the originally infecting Prophage or Prophages, again with Secondary Infection defined in this case in a Biomedical Sense.

Blocks on Secondary Infection—Biomedical Sense

In addition to a single bacterium being unable to support more than a single Burst, subsequently Adsorbing phages to that bacterium also and distinctly may fail to contribute genetically to the virion progeny of the phage infection. This is due to expression by phage infections of mechanisms of superinfection exclusion, as well as Superinfection Immunity. These terms, as defined here, are blocks to Secondary Infection at the level of the cell envelope (exclusion) and blocks at the level of the cell cytoplasm (Immunity) [63]. Not all Secondary Infections, in this Biomedical Sense, thus succeed in contributing genetically to the next generation.

This issue of phage genetic survival is likely less relevant to Phage Therapy than that Secondary phages (Biomedical Sense) otherwise will fail to give rise to Bursts of their own (previous subsection). That is, it is not a question of to what degree secondarily Adsorbing phages fail to contribute to the next phage generation that is important to Phage Therapy so much as that these secondarily Adsorbing phages essentially do not give rise to Bactericidal nor Productive Infections. That is, since they are Adsorbing to bacteria which already are being subject to Bactericidal Infections or Productive Infections (i.e., as effected by Primary Infections, Biomedical Sense). Mechanisms of superinfection exclusion therefore, I would argue, are not terribly relevant to Phage Therapy unless, as expressed by Prophages, they prevent treatment

phages from infecting Target Bacteria at all (i.e., as a form of Resistance to phages). This is similarly the case for Superinfection Immunity, though in that case it also would be only Temperate treatment phages which would be affected as mechanisms of Immunity generally do not impact infections by Strictly Lytic phages.

Single-Hit Killing Kinetics

Single-Hit Killing Kinetics refers to the fact that generally only a single phage must Adsorb to a bacterium to result in the killing of that bacterium, or at least this occurs to the extent that those adsorptions result either in Lytic Cycles or Abortive Infections, i.e., Bactericidal Infections. Single-Hit Killing Kinetics contrasts with the action of most antibiotics where individual bacteria generally must be exposed to numerous (such as thousands of) individual antibiotic functional units (i.e., individual molecules) to result in significant antibacterial action (thus, multi-hit kinetics). For discussion of Single-Hit Killing Kinetics and their pharmacological consequences, see Bull and Roland [344].

The utility of Single-Hit Killing Kinetics for Phage Therapy, though relevant as it means that only a single phage must reach a bacterium to result in that bacterium's death, versus, e.g., thousands of phages, nevertheless can be misleading. This is particularly as a consequence of phage Adsorptions being distributed Poissonally rather than evenly over Adsorbed bacteria. That is, it generaly actually *does* require multiple bacterial adsorptions – on average to individual bacteria, i.e., Multiplicities of Infection (MOI_{actual}) of somewhat greater than one – to result in multi-log reductions in numbers of viable bacteria. Thus while individual phages display Single-Hit Killing Kinetics, the aim with Phage Therapy nevertheless usually is to achieve multiple phage 'hits' (Adsorptions) per bacterium targeted, whether those phages are supplied directly by dosing or instead are present *In Situ* as a consequence of phage Population Growth (Auto Dosing).

Single-Step Growth

See One-Step Growth.

Specificity

See Host Range.

Spot/Spotting

Spotting refers to the application of small liquid suspensions phages, e.g., 10 µl, onto an already-initiated bacterial Lawn. A Spot may or may not result, depending in part on the number of Phage Particles applied along with the susceptibility of the bacterial strain to the applied phages. When high numbers of phages are applied, resulting in a clearing that is at least the size of the initially added phage suspension, then for the sake of avoiding ambiguity that Spot should never be described as a Plaque.

Two approaches to Spotting exist, those that employ lower numbers of Plaque-forming units (PFUs) and those that employ higher numbers of either PFUs or otherwise bactericidal Phage Particles. Spotting in the 'High-PFU' form most commonly is used as a means of inferring a phage's Host Range, but toward this end can be prone to false positives, i.e., which is clearing observed despite a phage otherwise displaying poor infection capabilities on a given bacterial host [345]. 'Low-FPU' Spotting for Host Range determination [196], by contrast, is not prone to false positives but, like Plaquing in general, can be prone to false negatives, that is, a failure to form Plaques even for some phage's which otherwise can display Productive Infections, such as due to phages displaying a low Infection Vigor (compare, that is, Efficiency of Plating with Efficiency of Center of Infection). Publications, however, do not always distinguish between these approaches, High- versus Low-PFU Spotting, when discussing Spotting.

Spot/Spotting—Low-PFU Spotting (Drop Plaque Method)

Low-PFU Spotting is simply a more spatially compact approach to generating phage Plaques (where, as noted, phage Plaques are not equivalent to phage Spots). To achieve Low-PFU Spotting, as with Plaquing generally, then Confluent Lysis is to be avoided. See Carlson and Miller [195], Carlson [261], Mazzocco *et al.* [346], and Letarov and Kulikov [197] for protocols. With Low-PFU Spotting, the number of Plaques which will give rise to declarations of too numerous to count, i.e., TNTC [307], will tend to be lower versus when the full area of a Petri dish is used for Plaquing. Alternatively, however, more individual Plaque assays can be done per Petri dish with Low-PFU Spotting.

Carlson and Miller [195] describe the procedure of Low-PFU Spotting as only "semiquantitative", presumably due to a tendency for Plaques to be present in numbers which technically are too few to count (TFTC). That is, due to the small size of the area which is phage-inoculated when Spotting, versus the area of whole Petri dishes, Plaque counts in the range of 30 to 50 (as typical cut offs for TFTC) will result in much greater Plaque crowding, potentially resulting in counts which effectively are TNTC even without actually exceeding TFTC thresholds. In addition, Carlson and Miller note that (pp. 428-429, emphasis mine), "The number of Plaques in a spot allows the calculation of an *approximate* Titer, which can be verified by appropriate plating." See also Carlson [261].

Kutter [196] provides a protocol for exploring phage Host Range by combining Low-PFU Spotting, Efficiency of Plating, and High-PFU Spotting.

Spot/Spotting—High-PFU Spotting

Unlike Low-PFU Spotting, High-PFU Spotting substantially contrasts with Plaquing. First, the resulting spots, as Confluently Lysed or simply fully cleared areas of bacterial Lawn, are as noted not themselves individual Plaques. Second, the lawn clearing observed may not even involve Plaque formation as it could be a consequence either of killing of bacteria via phage infection very early during Lawn formation (e.g., prior to any bacterial replication) or, especially given application of Lysates versus more purified phages, instead can be due to the action of bacterial antagonists that are other than phages, e.g., such as bacteriocins [347]. Only viable phages, however, will give rise to Plaques upon further dilution, i.e., as seen with Low-PFU Spotting.

Note that resulting spots should never be described as being due to Lysis from Without unless further characterization is undertaken so as to confirm that actual Lysis from Without has occurred. Nonetheless, the term Lysis from Without is often used in this context to describe the mechanistic underpinnings of Spot formation, e.g., [195,197]. This latter tendency likely is a consequence, as seen in many publications, of assumptions that the application of large numbers of phages to bacteria generally will tend to result in a Lysis from Without. However, not only is evidence for Lysis from Without in such instances almost universally lacking (though not so for phage T4, as specifically being considered by Carlson and Miller), but in fact Spots can form even given initial phage Multiplicities of Infection, in this case, MOI_{input}, of less than one.

Strictly Lytic

Strictly Lytic is a description of a phage which releases virions Lytically (virion Release) and also is not Temperate. The term Obligately Lytic is used equivalently, as too also is Virulent (as Strictly Lytic) and one also sees ‘exclusively lytic’. Professionally Lytic phages in turn represent a subset of Strictly Lytic phages. Strictly Lytic phages tend to be preferable for Phage Therapy purposes to Temperate phages, while Professionally Lytic phages as a subset of Strictly Lytic phages are arguably even more appropriate.

‘Lytic’ (used unqualified) as a Synonym?

Note that many publications seem to use the term Lytic in an unqualified manner as a synonym for Strictly Lytic. This is unfortunate as most Temperate phages also are Lytic Phages, thus often making it difficult to distinguish ‘Lytic’ meaning all phages which Release virions Lytically (which would include most Temperate phages, e.g., phage λ) or

instead 'Lytic' meaning only those phages which are Strictly Lytic. It can be difficult, that is, to tell whether or not the intention in publications is to include Temperate phages as typically 'Lytic Phages' or instead to exclude such phages [280]. There is utility, as a consequence, in qualifying the term Lytic when describing phages: if the intention is that of Strictly Lytic, then it or one of its synonyms should be employed rather than simply 'Lytic'. If the intention instead is *not* just Strictly Lytic, then that ought to be mentioned as well, e.g., 'all functional tailed phages are lytic, whether Temperate or not'.

The term Strictly Lytic also can be used to describe the properties of infections rather than phages themselves. Thus for example is "Strictly lytic infection cycle" [348], with a meaning which I equate with purely lytic infection as considered above (see Lytic Infection—Purely Lytic Infection).

Sur Mesure

From Pirnay *et al.* [130], literally meaning 'custom-made', or less literally, 'bespoke', Sur Mesure refers to customized phage Formulated Products which are designed to be applicable to the needs of specific patients. Particularly, Sur Mesure can be viewed as a form of personalized Phage Therapy. See also Autophage and Phage Bank. Contrast with Prêt-à-Porter.

Synergy

The concept of Synergy should be used to refer to greater than additive effects, that is, 'greater than the sum of the parts'. This term is used often in the Phage Therapy literature, but not necessarily always as consistent with the above definition. Instead, Synergy may be equated with simply additive or non-antagonistic effects. Strictly speaking, however, with Synergistic interactions between two distinct entities, e.g., two phages or a phage and an antibiotic (i.e., as during Combination Therapy), then greater levels of effects should be observed than would be expected based on the activities displayed by each when acting alone. It is important, however, to recognize that Synergistic interactions between antibacterial agents is not essential for Combination Therapies as observed gains in efficacy will remain gains efficacy even if they are not necessarily synergistic.

Facilitation, Antagonism, Tolerance, Resistance, Ecology, and Evolution

If each phage alone were able to produce 100-fold reductions in bacterial densities, then a 10,000-fold reduction in bacterial density upon administration of both phages would *not* represent a synergistic interaction between the two phages, but

instead an only additive interaction (100-fold reductions by one phage and then 100-fold reductions by the other, with 100 × 100 = 10,000). On the other hand, only 100-fold reductions would not necessarily represent antagonistic interactions, but instead only a lack of additive interactions, i.e., the two phages may simply be targeting the same bacterial subpopulation in the same way. Chaudhry *et al.* [176] would describe, e.g., 1,000-fold killing in this example as "Facilitation", which would be less than additive but still greater killing than seen upon use of only one of the antagonists. Alternatively, 100,000-fold reductions upon application of these two phages together, i.e., as greater than 100 × 100, certainly would be suggestive of Synergistic bactericidal interactions.

The concepts of Synergy, additive interactions, antagonistic interactions, or facilitation, as used here, refer to the combined properties of two or more antibacterial agents. Resistance as well as Tolerance, by contrast, are properties of bacteria or bacterial infections of a host (one such as ourselves) rather than properties specifically of antibacterial agents. Synergy among antibacterial agents nevertheless will tend to be measured in terms of degrees of retention by bacteria of such Resistance or Tolerance. We can also consider, as I do below, Synergy in Phage Therapy as ecological versus evolutionary concepts, both of which will impact Phage Therapy, but in different ways.

Synergy—Ecological Synergy

From the perspective of bacterial sensitivity to phages, ecological issues could be viewed as ones of phenotypic bacterial infection *Tolerance* to Phage Therapy. Especially this is *In Situ* interference by infecting bacteria to phage action which is not necessarily similarly observed *In Vitro*, and which does not involve changes in the genotype of Target Bacteria. With Ecological Synergy, the issues thus are more or less independent of the evolution of genetic phage Resistance by Target Bacteria, but instead are a function of environmental conditions affecting bacterial sensitivity to antibacterial agents, that is, as a function of their ecology. For instance, one phage could be effective at allowing the other phage to reach biofilm bacteria, but not at killing those bacteria, while a second phage could be effective at killing bacteria once it has succeeded in reaching them, but not at reaching the bacteria on its own. The result in combination could be somewhat more killing of otherwise genetically identical bacteria than would have been readily anticipated based on the killing ability of the two individual phages as observed in isolation.

Ecological Synergy thus is a function of the ability of combinations of phages to interact with, kill, and potentially also propagate in association with otherwise phage-sensitive bacteria. Here bacterial sensitivity to phages may be defined variously, e.g., see the previous paragraph where bacteria are sensitive to the two different phages, but in different ways. Thus, with Ecological Synergy the ability of two phages to control

an otherwise genetically static bacterial population is a greater than their sum-of-the-parts ability to overcome a bacterial infection's Tolerance to Phage Therapy. Similarly, this could be Synergy between phages and antibiotics in overcoming a bacterial infection's combined Tolerance to both phages and antibiotic. For example, a phage, perhaps by partially disrupting a biofilm, may increase an infection's sensitivity to an antibiotic, thus resulting in overall greater antibiotic-mediated killing in combination with otherwise unchanging phage-mediated antibacterial activity.

Synergy—Evolutionary Synergy

Issues pertaining to bacterial acquisition of ***Resistance*** to phages would be ones involving changes to bacterial genotype, rather than solely changes to bacterial phenotype. These therefore are evolutionary in their nature rather than ecological, i.e., 'evolutionary' synergy [176]. Nevertheless, and as noted, Synergy itself is not a bacterial property, though nonetheless can be measured in terms of degrees of bacterial Resistance, or Tolerance, that persist in the face of combined antibacterial action (Combination Therapy). The issue thus is one of evolutionary acquisition by Target Bacteria of Resistance to phages, as well as potentially resistance to antibiotics, with Evolutionary Synergy a function of the degree to which two or more bacterial antagonists when used in combination are able to lower, more than expected, the potential for evolution of bacteria-mediated Resistance to those agents. Note that a narrower version of this idea of Evolutionary Synergy is provided by Chaudhry *et al.* [176] and see also [350].

If mutation to Resistance occurs at some rate to each of two antagonists and Resistance to both occurs at a rate that is a multiple of the two individual rates, e.g., $10^{-4} \times 10^{-4} = 10^{-8}$, then that is only an additive interaction. A combined rate of dual mutation-to-Resistance of 10^{-9} – which is a *lower* than the expected rate of bacterial mutation to Resistance as based on rates of mutation to Resistance to each entity alone – would by contrast represent an Evolutionary Synergistic interaction between the two antibacterial agents. Such Synergy could be a result of potentially co-occurring bacterial Resistance mutations having negative epistatic effects on bacterial functionality. For example, this could be were two mutations co-occurring together in the same bacterium to result in bacterial death [351], but with no resulting bacterial death were either mutation instead present alone (such as the knocking out the activity of two otherwise functionally essential but redundant bacterial surface proteins). Thus, observation of dual mutations-to-Resistance would occur at a lower than expected rate since some fraction of these bacterial mutants would not be viable, which from the perspective of the combined bacterial antagonists would be a Synergistic interaction.

On the other hand, rates of dual mutation-to-Resistance by bacteria of greater than 10^{-8} in this example, e.g., 10^{-6}, could imply some degree of Cross Resistance to the two entities occurring per bacterial mutation, i.e., a pleiotropic effect. From the perspective of the two antagonists this would *not* represent a positive Evolutionary Synergistic impact of the two agents on bacterial survival. Nevertheless, we could describe this as an example of combined evolutionary facilitation.

Tailocin

See High Molecular Weight Bacteriocin.

Target Bacterium (Target Bacteria)

Target Bacterium refers to the organism that is being directly pursued during Phage Therapy. Ideally that bacterial strain will be susceptible, by treatment phages, to Bactericidal Infections (for Passive Treatment), and also to Productive Infections (for Active Treatment). Ideally as well, Target Bacteria will be physically reachable by intact Phage Particles (Penetration). By employing phage Cocktails as Formulated Products, the number of possible Target Bacteria can be expanded to include not just a diversity of bacterial strains within a single bacterial species but even a diversity species or genera of Target Bacteria.

Bacteria also may be inadvertently targeted, though this presumably is less of an issue the less that treatment phages interact with normal microbiota during use. The latter could be due to treatment phages possessing relatively narrow Host Ranges, and could also be due to treatment simply of more contained infections, e.g., skin wounds, or within what otherwise would be sterile body locations, such as treatment of bacteremias. Well-contained treatments, that is, should limit physical phage exposure to non-Target Bacteria.

Temperate

Temperate refers to phages which are capable of displaying latent infections, that is, Lysogenic Cycles. The term 'Lysogenic', however, should not be substituted for 'Temperate', as in 'Lysogenic phage' to mean Temperate phage, as discussed in the following paragraph. In terms of Phage Therapy, generally Temperate phages should be avoided as therapeutic agents unless alternatives, i.e., Strictly Lytic phages, are highly difficult to obtain, or to generate. For a review on Temperate phages, see [352].

Most Temperate Phages are also Lytic Phages

There appears to be a tendency in publications to use simply 'Lytic' to contrast with Temperate when describing especially hypothetical phages for phage therapy use. This substitution is incorrect, however, as most Temperate phages, e.g., phage λ, are also clearly Lytic Phages as well. The origin of this error likely comes from incorrectly substituting 'Lysogenic' for 'Temperate' when referring to types of phages (previous paragraph) in combination with introductory textbooks correctly contrasting Lytic *Cycles* with Lysogenic *Cycles*. In those textbooks, however, this distinction is in terms of infection aspects, i.e., types of infection cycles, and this is rather than in terms of overall phage properties. Instead, it is Obligately Lytic, Strictly Lytic, Professionally Lytic, or Virulent (as Strictly Lytic) phages which should be contrasted with Temperate phages [280]. More generally – thereby including non-Lytic Phages as well – contrast Temperate with obligately, strictly, or professionally *productive,* i.e., see Productive Infection.

Titer

Titer refers to the number of phages – or more generally, number of Virus Particles – as found per unit volume of a fluid. Generally volume is presented in milliliters or, equivalently, in cubic centimeters, with phage numbers often presented as Plaque-forming units (PFUs). The titer associated with phage Formulated Products should always be unambiguously indicated in publications for every phage type present, e.g., X PFUs/ml for phage A, Y PFUs/ml for phage B, etc. This contrasts with more ambiguous wording, forcing readers to do these calculations themselves (i.e., when only indicating Titers present *prior* to mixing), or omitting Titer measures altogether (as is commonly seen when Multiplicity of Infection is presented to describe phage doses instead). See Abedon [353] for an online phage Titer calculator.

In Situ *and* Ex Situ *Phage Titers*

It can be useful to keep track of phage Titers that are present *In Situ* in the course of Phage Therapy experiments, as this is a key determinant of the phage potential to impact Target Bacteria and also represents the key phage dosing end point. This is true even though under more complex circumstances it may be difficult to distinguish Virus Particles, that is, Free Phages, from phage-infected bacteria in terms of PFUs. Free Phages and phage-infected bacteria, as may be described collectively as infective centers, in other words can both initiate Plaques. Nevertheless, if phage titers *In Situ* can be ascertained, e.g., such as in terms of serum titers, or as may be determined following biopsies or animal sacrifice, then this information ought to be obtained even if Free

Phages are not distinguished from infected bacteria, as *In Situ* phage Titers represent a key pharmacokinetic measure.

It is important during Phage Therapy experiments to also be aware of the Titers of phages that are present during the course of bacterial enumeration, as *ex situ* phage Adsorption can result in artificial declines in bacterial densities [236-238]. The greater phage Titers are in the presence of bacteria during enumeration, then the greater such potential losses. Though this latter problem can be countered via sufficient dilution in the course of disrupting *In Situ* structures (e.g., solid tissues or biofilms) and/or use of phage- but not bacteria-inactivating agents (i.e., virucides), it is important nevertheless to provide empirical evidence, or at least calculations (see Killing Titer), indicating that phages are not reaching bacteria in large numbers during enumeration. This is versus merely assuming that *ex situ* phage Titers are not an issue, or instead indicating only that it was not found to be an issue for others, since *ex situ* declines in bacterial numbers, versus *In Situ*, would contribute to a Phage Therapy efficacy false positive results.

Tolerance

Tolerance describes phenotypic interference by a bacterial infection with the actions of an antibacterial agent. Specifically, while bacterial sensitivity is observed in the laboratory, i.e., *In Vitro*, with Tolerance it is observed to a lesser extent *In Situ*, holding bacterial genotype constant. This concept is seen with antibiotics and typically is as associated with bacterial persister cells, which display a physiological rather than a mutational reduction in sensitivity to an antibiotic [354-358]. Contrast Tolerance with Resistance, and see also the concept of ecological synergy (Synergy—Ecological Synergy).

Generally infection Tolerance is associated with biofilm formation by bacteria, though can as well involve bacteria location, such as within poorly vascularized tissues. Furthermore, Tolerance of bacterial infections to Phage Therapy is even less well understood than Tolerance of bacterial infections to antibiotics, but conceivably can be a relevant factor given Phage Therapy failures. For a review considering both phage Tolerance and phage Resistance, see [333].

Translocation (Transcytosis)

Bacteriophage Translocation is movement of Phage Particles across especially intestinal mucosa [359,360]. This can serve as a route of phage delivery to internal organs including via *per os* dosing or instead via rectal delivery [361]. *Per os* dosing also,

of course can be used to target gastrointestinal bacteria directly [362]. Note that the term Translocation can also be used to describe phage nucleic acid movement into the bacterial cytoplasm given phage virion Attachment/Adsorption. Transcytosis refers to a specific mechanism of vesicle-mediated movement of materials from one side of a eukaryotic cell to the other, and represents one possible mechanism of bacteriophage Translocation [363,364].

Turbid Plaque

See and contrast with Clear Plaque.

Transduction

Transduction is virion-mediated movement of non-viral DNA from one cell to another. Usually this movement will be differentiated into a specialized transduction versus a generalized Transduction. These latter concepts can be distinguished especially in terms of the presence or absence of virus DNA within transducing Virus Particles, along with the presence of non-viral DNA (the latter the transduced DNA). With specialized transduction, virus DNA is present within the transducing particle (a phage virion) along with the transduced DNA (but the latter in relatively small quantities), whereas with generalized transduction virus DNA is *not* also present within the transducing particle while transduced DNA is present in relatively large quantities. See Schneider [365] for a recent review of phage-mediated Transduction.

Specialized transduction is normally considered to be a property of Temperate phages rather than of Strictly Lytic phages. Also associated with the concept of specialized transduction is that of phage morons, standing for ‘more DNA’ and especially referring to non-viral DNA that has been relatively newly integrated into functional phage genomes. Consider also Lysogenic Conversion. With regard to Strictly Lytic along with Temperate phages, it is generalized transduction especially which is considered to be a possible concern as this could result in the transfer of large quantities of DNA from pathogenic bacteria to non- or less-pathogenic bacteria, such as from Phage Therapy Targeted Bacteria to otherwise bystander commensal bacteria.

Treatment Resistance

Bacterial Resistance to phages that arises in the course of Phage Therapies. Contrast with both Community Resistance and phage Tolerance. See [28].

Virulent

With regard to phages, the concept of Virulence has at least four meanings. Phages, in particular, can be Virulent in the sense that they are not able to Lysogenize (Strictly Lytic as Virulent as well as Temperate Phage Mutant as Virulent), because they are highly effective at eradicating populations of Target Bacteria (Damaging to Bacteria as Virulent), or because they can encode bacterial virulence factors (Contributing to Bacterial Virulence). All four perspectives can be relevant to Phage Therapy, though meaning typically must be inferred from context.

Virulent—Strictly Lytic as Virulent

Generally the most common usage of Virulent for modern Phage Therapy is that of Virulent as a synonym for Strictly Lytic, contrasting Temperate [280]. Strictly Lytic phages generally are preferred over Temperate phages for Phage Therapy.

Virulent—Temperate Phage Mutant as Virulent

Certain Lysogenic Cycle-defective mutants of Temperate phages are described as Virulent. These are Clear Plaque mutants which are able to grow on bacteria Lysogenized by their parent Temperate phage [249]. Such Virulent mutants are also Strictly Lytic, but are not Professionally Lytic.

Virulent—Damaging to Bacteria as Virulent

The oldest of the concepts of phage Virulence, though one related to the first two (i.e., Strictly Lytic as Virulent and Temperate Phage Mutant as Virulent), is to describe as Virulent those phages which are highly effective at eradicating Target Bacteria, e.g., Smith and Huggins [366]. This antibacterial phage Virulence may be observed particularly in terms of the lysing of broth cultures of bacteria (Culture Lysis) but as also may be seen within the context of Plaque turbidity (see Clear Plaques).

The relationship of this third concept to the first two is that Temperate phages, due to their display of Lysogenic Cycles, can be less effective than Strictly Lytic phages at eradicating Target Bacteria, such as in broth cultures (especially as viewed after overnight incubation) or, at least in principle, during Phage Therapy. In any case, this third concept of phage Virulence is equivalent to definitions of pathogen Virulence more generally, that is, capacity to harm affected organisms, where here the phage is serving as the pathogen and the Target Bacterium, or its culture, is serving as the affected organism.

Virulent—Contributing to Bacterial Virulence

This is Virulence referring to the phage potential, especially for Temperate phages, to encode bacterial virulence factors and thereby contribute to bacteria-caused disease [274,275]. This usage generally would be within a context of Lysogenic Conversion.

Virus Particle

Equivalent here to Phage Particle.

Conclusion

A mutually common set of terminology possessing equivalent meanings is essential for effective communication. As an approximately one hundred-year-old discipline, phage therapy along with phage biology more generally have accumulated a number of such terms, not all of which are consistently unambiguously employed. Here I have attempted to clarify the meaning of over 100 of these terms. It is my hope, at a minimum, that this effort promotes awareness of issues of ambiguous usage, but also that it might stimulate robust discussion as well as increased appreciation of the importance of many of these terms toward further development of the techniques of phage therapy.

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