Universitat Autònoma de Barcelona

BACTERIOPHAGE DETECTION AND QUANTIFICATION IN TRANSPARENT AND TURBID SAMPLES

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BACTERIOPHAGE DETECTION AND QUANTIFICATION IN TRANSPARENT AND TURBID SAMPLES

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A thesis submitted by Denis Rajnović entitled **Bacteriophage detection and quantification in transparent and turbid samples**

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To my family, friends, labmates and mentor who unconditionally believed, supported and guided me through this hard, but wonderful period.

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List of abbreviations

AC	alternating current
AK	adenylate kinase
ATP	adenosine triphosphate
BAVS	Bacterial and Archaeal Viruses Subcommittee
CNT	carbon nanotube
CTC	5-cyano-2,3-ditolyl tetrazolium chloride
DAL	double agar layer
DC	direct current
ddPCR	droplet digital PCR
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EMA	ethidium monoazide
EPD	end point of detection
LFA	lateral flow assay
LPFG	long-period fiber gratings
gLAMP	in-gel loop-mediated isothermal amplification
ICTV	International Committee on Taxonomy of Viruses
INT	1-(4-Iodophenyl)-5-(4-nitrophenyl)-3-phenylformazan
IPTG	isopropyl β-d-1-thiogalactopyranoside
LB	Luria Bertani medium
LOD	limit of detection
L. bulgaricus	*Lactobacillus bulgaricus*
M. aeruginosa	*Microcystis aeruginosa*
MALDI-ToF	Matrix-Assisted Laser Desorption-Ionisation - Time of Flight
MmC	mitomycin C
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	not available
NanoIDA	nano interdigitated array
NTA	nanoparticle tracking analysis
OFRR	opto-fluidic micro-ring resonator

P. syringae	*Pseudomonas syringae*
PB	phosphate buffer
PCR	polymer chain reaction
Ppy	polypyrrole
PEG	polyethylene glycol
PI	percentage of inhibition
PMA	propidium monoazide
PT	phosphotungstic acid
QCM	quartz crystal microbalance
qPCR	quantitative polymer chain reaction
RNA	ribonucleic acid
RT-PCR	reverse transcription polymer chain reaction
SAM	self-assembled monolayers
SB	super broth
SPD	start point of detection
SPR	surface plasmon resonance
S. thermophilus	*Streptococcus thermophilus*
STEC	shiga toxin-producing *Escherichia coli*
Stx	shiga toxin
ToD	time of detection
TTC	2,3,5-triphenyl-2H-tetrazolium chloride
UA	uranyl acetate
UHT	ultra heat treated
UV/VIS	ultraviolet/visible
WST-1	water soluble tetrazolium salts
XTT	2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide

List of units

%	percentage
°C	degree Celsius
A	area of the curve
c	concentration
cfu	colony forming unit

Da, kDa	daltons, kilodaltons
e	Euler’s number
fg	femtogram
gc	gene copy
MW	molecular weight
MWCO	molecular weight cut off
N	number
NTU	nephelometric turbidity units
OD_{600}	optical density 600nm wavelength
pfu	plaque forming unit
rcf	relative centrifugal force
RFU	relative fluorescence unit
rpm	rounds per minute
V	volume

Summary

Most of the phage detection and quantification techniques are developed for applications in public health and industry environments, and for many years conventional methods like Double Agar Layer and Electron Microscopy were the only available option to detect and quantify phages. These methods are either labour intensive and time consuming or require expensive equipment, respectively. However, this has changed with the introduction of novel technologies that brings new solutions and alternatives to conventional methods. In order to build an effective tool, phage detection methods must take into account several factors: 1) type of phage; 2) level of sensitivity required; 3) availability of cultivable hosts; 4) possible interference by the sample matrix; 5) availability of sophisticated equipment and/or highly skilled personnel and, conditioning most of the above, 6) type of application (phage therapy, bio-control studies, food fermentation industry or environmental monitoring).

The work developed in this thesis explores the possibility of carrying out sensitive detection and quantification of specific phages, by analyzing the dynamic behavior of the phage-host system. The dataset has been published and made available to the community as it constitutes probably the first detailed study on phage-host concentration dependence that has been published from the discovery of bacteriophages in the 1920's. All of these elements can be used as the basis for the development of specific methods tailored to any of the areas mentioned above.

In the first phase we have monitored optical density (OD) changes during phage-induced culture lysis in clear media in order to evaluate phage-host kinetics over 90 different combinations of bacteria/phage concentrations. In a second phase the fluorescent properties of the redox dye resazurin have been employed to overcome the problems observed when attempting to perform optical density kinetic measurements in media of high turbidity. Thanks to the fluorescent properties of resazurin, the phage-host kinetics of 186 different combinations of bacteria/phage concentrations have been evaluated in synthetic turbid media as well as in a high complexity matrix such as milk. Both OD and fluorescence-based approaches are complementary and have similar LOD and Time of Detection ($5x10^1$ pfu/mL in 3.5 h). OD-based assay is quantitative, and the

resazurin assay is semi-quantitative but faster, detecting high phage numbers in less than 60 min. The working principle of both assays is highly flexible and can be easily adjusted to any specific application by choosing the right phage-host combination whether it be in clean or turbid samples. Finally, the technology used is relatively simple and well suited for further miniaturization, automatization and high-throughput analysis.

1. INTRODUCTION

1.1 Discovery of bacteriophages

Bacteriophages, submicron viral particle that cause lysis in bacteria were discovered independently by Frederick Twort and Felix d'Herelle in 1915 and 1917, respectively (Taylor 2014, Vandamme and Mortelmans 2019, Gordillo Altamirano and Barr 2019). At the time, the cause of lysis was not completely understood and for years it stayed unclear whether bacterial lysis was a result of an autocatalytic enzyme or a virus. Twort's doubts can be best understood when in his research paper he writes…. *"[This] may be living protoplasm that forms no definite individuals or an enzyme with the power of growth… In any case, whatever explanation is accepted, the possibility of its being an ultra-microscopic virus has not been definitely disapproved because we do not know for certain the nature of such a virus…"* In addition, the attempt to isolate the lysing substance and to identify it under the microscope didn't bring any clarification either. In 1920's, d'Herelle with his published papers and books introduced the term bacteriophage, as well as plaque assay, phage activity dependence on temperature, and the procedures of phage isolation and purification, therefore becoming the founder father of bacteriophage studies. Not more than 20 years later the morphology of the "lysing substance " was clarified with first electron microscope (EM) micrographs of bacteriophage T7 obtained by Ruska in Germany during World War II (Ackermann 2011).It is reported that Felix d'Herelle was on his death bed when French scientist Hauduroy showed him a bacteriophage micrograph. Bacteriophage T7 belongs to T-phages, a serie of 7 virulent phages (T1-T7) which infect *E. coli*., and in this thesis the bacteriophage model used was T4. An original micrograph of bacteriophage T4 taken at the Autonomous University of Barcelona with electron microscope, is shown in Figure 1. With the development of EM, a taxonomical classification of phages based on morphology was possible and with a rate about of 100 phages examined per year, more than 6300 phages have been analyzed up to date (Ackermann 2013). Entering the omic's era and with the first complete DNA genome sequencing of phiX174 phage by Fred Sanger and his team in 1977 (Sanger et al. 1977), more complex taxonomical classification was made possible which is constantly evolving and maintained by International Committee on Taxonomy of Viruses

(ICTV)-Bacterial and Archaeal Viruses Subcommittee (BAVS) (Chibani et al. 2019). As can be seen in Figure 2, a total of 3700 phage genomes have been sequenced until now and the number is growing exponentially.

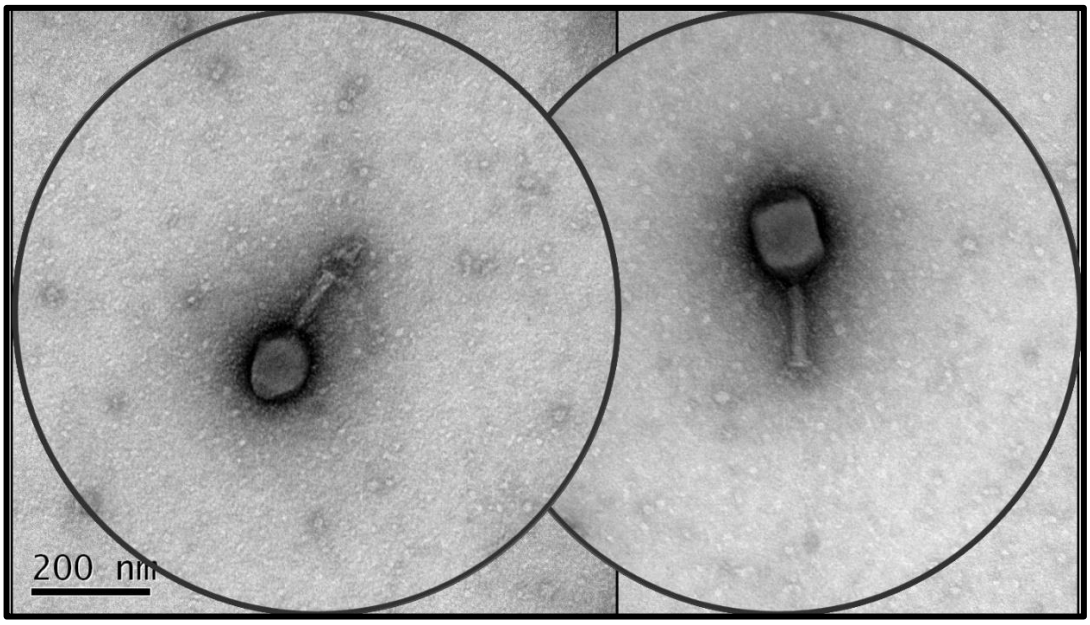

Figure 1. Transmission electron microscope (TEM) image of our T4 bacteriophage used in this thesis. T4 phage has a characteristic icosahedral head and a contractile tail with tail fibers. It is 90nm wide and 200 nm in length (original photograph by the author).

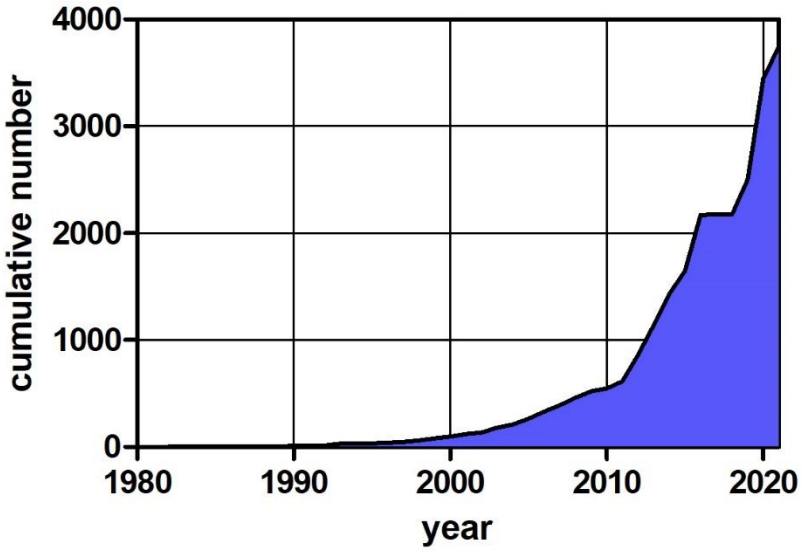

Figure 2. Cumulative number of sequenced phage genomes per year (https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?taxid=10239&host=bacteria

From a quantitative point of view phages are the most ubiquitous organism on the planet with an estimated total number of 10^{31} viral particle (Hendrix et al. 1999, Mushegian 2020). Due to their relevance in microbial ecosystem dynamics, as well as their impact in industry and in public health, detection and quantification of known phages proves to be of human and ecological importance and it will continue to rise as long as new phages and methods are discovered.

1.2 The importance of phage detection

1.2.1 Public Health

One of the first ideas proposed by Felix d'Herelle back in the 1920's, was to use the lytic properties of bacteriophages in order to combat pathogenic bacteria (Taylor 2014, Vandamme and Mortelmans 2019). This concept, known as phage therapy, was mainly suppressed during the last century thanks to the efficiency of antibiotics (Gordillo Altamirano and Barr 2019). However, the emergence of antibiotic resistant strains, and the lack of interest of pharma companies in the development of new antibiotics have stimulated interest in phage therapy once again. Either way, this idea was never left in ex-soviet republics where phage therapy was implemented from 1940's onwards, however the publications in this field were mainly written in local languages which makes them difficult to assess and interpret for the scientific community (Taylor 2014, Vandamme and Mortelmans 2019, Gordillo Altamirano and Barr 2019). In terms of satisfying strict international scientific criteria's and laws, for instance in Poland, the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy (HIIET) over 2000 patients have been treated with a success rate of 30%-90%. The data and reports available from these trials constitute one of the best documented cases of phage therapy up to date (Vandamme and Mortelmans 2019). At the moment, many international research groups are involved in phage therapy studies that attempt to provide solutions in fields like cystic fibrosis, tooth decay biocontrol and respiratory tract, gastrointestinal and dermal infections (Harada et al. 2018, El Haddad et al. 2019). In this area fast phage quantification methods are required to study phage virulence, host susceptibility, phage kinetics and dynamics and others (Mutti and Corsini 2019, Malik et al. 2017, Hyman 2019). Another important aspect of bacteriophages in public health is their possible interference with microbial diagnostic tools (Brown-Jaque et al. 2016). It has been reported that phages have been isolated from clinical samples such as blood, urine, ascites fluid etc (Navarro and Muniesa 2017). In these samples, phages can present a burden when isolating pathogenic bacteria of interest during culture enrichment step, affecting and reducing total colony forming unit count, and preventing confluent bacterial growth necessary for antibiogram tests. In environmental diagnostics, bacteriophages are either used as surrogates for human pathogenic

viruses to assess the efficiency of water treatment processes (Wu et al. 2017), or as indicators of fecal contamination (McMinn et al. 2017, Jofre et al. 2016). In addition, the presence of certain phages in fresh meats, shellfish, vegetables and processed meats (Hsu et al. 2002, Kennedy et al. 1986) has been related to fecal contamination of food, thus enabling their use as a tool in food safety management. According to water quality regulations and guidelines for different types of drinking water (European commission 2017, World Health Organization 2017) requires absence of coliphages in 100mL water samples. Therefore, sensitive methods able to detect 1 pfu per 100ml are required and the performance of the method should not be affected by the compounds usually present in water samples.

1.2.2 Industry

The microbe-based fermentation processes have been familiar to humankind since prehistory. With the appearance of large-scale industrial mass production of fermented products, the interference due to phages must be maintained at minimal levels. Phage contamination originating in air, bacterial strains, raw ingredients, equipment and surfaces etc (Samson and Moineau 2013) has been reported in dairy (Marco et al. 2012), acetone-butanol (Jones et al. 2000), 1,3 propanediol (Halter and Zahn 2018), fermented soybean (Nagai 2012), wine (Doria et al. 2013), antibiotic and enzyme, vinegar production and many other (Vandamme and Mortelmans 2019). In these processes phages are responsible for complete loss of the production batch, or a loss of quality of the final product. To prevent the failure of microbe-based processes, sensitive and fast detection methods must be implemented. These methods should be able to perform real or near real time monitoring in matrices of different complexities. From the point of view of food safety, phages have been used as a potent-alternative for biocontrol, bio-preservation and bio-sanitation applications against spoilage and pathogenic bacteria. Their efficiency in animal, crop and water treatment, food preservation, as well as in sanitation of equipment and surfaces has been proved in numerous studies (Vandamme and Mortelmans 2019, Harada et al. 2018, Sillankorva et al. 2012, Endersen et al. 2014). In 2006, the US FDA approved a phage preparation for food preservation purposes as a safe food additive for human consumption (FDA 2006).

1.3. Conventional methods of phage detection

1.3.1 Double Agar Layer

The oldest and the most established technique in phage laboratories around the world is the Double Agar Layer or DAL method. This method was first described by Gratia in 1936 (Gratia 1936) and later popularized by Adams in 1959 (Adams 1959). In brief, the method consists of a short incubation of bacteriophages and host bacteria in culture media, then mixing it with warm liquid agar (soft/top agar) and pouring it onto an agar plate eventually making a double layer of agar. In the absence of phages a continuous bacteria lawn is formed, while in the presence of phages, small clear areas of lysis (known as plaques) can be observed in the bacteria lawn. With time, more detailed and sophisticated variations of DAL have emerged (Kropinski et al. 2009, Lillehaug 1997, Sambrook and Russell 2001). The method can be further modified into a spot test (Mirzaei and Nilsson 2015, Kutter 2009) which makes it more simple, cheaper and faster with higher throughput power, but at the expense of overall sensitivity. Either way, the formation and sizes of the plaques, displayed as clear zones in the bacteria lawn, depends on factors such as growth conditions (top soft agar concentration e.g.), the phage (lysis time, e.g.) and the host (lawn density e.g.) all of which must be considered in terms of performance (Gallet et al. 2011, Mullan 2002). The limit of detection (LOD) of this method is 10^0 plaque forming units (pfu) per mL and currently is one of the most sensitive methods available (Hagedorn et al. 2011, Blanch et al. 2020). In addition of being used for enumeration, the DAL method is used for phage and phage mutant isolation, characterization of plaque morphology, etc. The time required to obtain the results is 18-24 h or more depending on the host strain. The method is time-consuming, and is labor extensive, with many dilution steps and replicas involved, especially if the concentration range is unknown and many samples are processed at once. In addition, the impossibility of converting it into an automatized or semi- automatized format for high-throughput applications makes the DAL method ill-suited to provide solutions for current challenges and applications.

1.3.2 Microscopy

The invention of electron microscopy (EM) helped to elucidate the bacteriophage structure in 1940's (Ackermann 2011), and up to date more than 6500 have been analyzed with this method (Ackermann 2013). Electron microscopy is a primary tool when phage morphology and size are into question, and it is also the first tool of choice when counting total free viruses in aquatic systems. The detection limit is relatively poor around 10^5 pfu/mL and the results can be obtained under 1h if additional sample pre-treatment and manipulations are not required (Zhang et al. 2013). The most popular staining method for studies of phage structure, is negative staining by phosphotungstic acid (PT) or uranyl acetate (UA), which is undisputedly the fastest staining technique available (Ackermann 2013). Although being an information-rich method, relatively expensive equipment, the need for trained personnel and poor throughput power makes it impractical for everyday implantation in environments where phage detection and monitoring are needed on a routine base. Fluorescence microscopy has replaced electron microscopy in aquatic phage research as a more affordable quantification method, in which fluorescent nucleic-acid specific dyes such as SYBR green are used to label and quantify phages (Breitbart et al. 2018). Although having similar detection limits, it has greater throughput power, and it is faster than EM.

1.4 State of the art

1.4.1 DNA-based methods

With the discovery of genome sequencing (Sanger et al. 1977) and polymer chain reaction (PCR) technology (Mullis et al. 1986) in the 70's and 80's, a completely new era of phage classification and characterization based on their metagenomes had begun. DNA based method are summarized in Table 1. Most if not all these methods rely on polymer chain reaction for detection. Although PCR is a well-established method for bacteria detection, this isn't the case with phages due to absence of universal target sequences by which different phages could be identified, therefore, missing a standardized protocol for their detection (Adriaenssens et al. 2014, Sullivan 2015). The detection limit of PCR method is usually in the range of 10^3 -10^5 pfu/mL and the time of the assay is never shorter than 2h. Before the assay, usually DNA/RNA extraction is performed, adding an extra 2h to the total length. In order to increase the sensitivity, pre-concentration steps like magnetic capture hybridization (Dupont et al. 2005) or PEG (Labrie and Moineau 2000) concentration may be used, at the expense of overall time. In most cases the matrix of the sample must be discarded due to presence of PCR inhibitors that lead to a decreased sensitivity or to false-negative results (Schrader et al. 2012), thus adding to the complexity of the assay. While conventional PCR provided only qualitative results, the development of quantitative PCR (qPCR) allows the monitoring of phage DNA amplification in real time, by following an increase in fluorescence intensity by a DNA intercalating dye. qPCR has improved both sensitivity and detection when compared to classical PCR, providing analytical solutions for the detection of lacto and coliphages in dairy, food, water, faecal, clinical and air samples, as shown in Table 1. Novel technologies based on the PCR principle like droplet digital PCR (ddPCR) and in-gel loop-mediated isothermal amplification (gLAMP) have shown higher resistance to the presence of inhibitors in the samples when compared to qPCR (Morella et al. 2018, Huang et al. 2018). This can be attributed to the working principles of the methods as well as to how samples are distributed and read in the reaction chambers. For instance, ddPCR, is an endpoint reading method in which a PCR reaction is partitioned into numerous individual reaction droplets and in the presence of low to moderate inhibition it can still provide

accurate quantification data without the need of a standard curve (Morella et al. 2018, Racki et al. 2014). In gLAMP, PCR reactions are done in the gel matrix, thus limiting the movement of inhibitors through the system (Huang et al. 2018). Miniaturization of the method and transfer of the technology to a "lab-on-a-chip platform" with smartphone imaging has allowed a reduction in the cost of the equipment while providing satisfactory sensitivity with a LOD of 10^3 pfu/mL in a 30 min time frame. In marine sciences, the identification and relative quantification of viral taxa in samples of seawater that contains significant degree of phage diversity, the methods of choice are, metagenomic analysis or qPCR in which degenerative primers are used to encompass genetic diversity (Baran et al. 2018). PCR technologies are valuable tools for phage identification without previous knowledge of the target host or when the host that cannot be grown in culture. In addition, these technologies can detect shiga-toxin (Stx) converting bacteriophages which are responsible for lateral gene transfer and the emergence of Stx producing *E. coli* (STEC) variants causing severe human diseases (Imamovic et al. 2010, Imamovic and Muniesa 2011). Unlike bacteria in which all species possess 16 rRNA genes, phages don't have a universal sequence that can be used for specie and genus identification (Clokie et al. 2011, Rohwer and Edwards 2002). Therefore, prior to developing a method, whole or partial genome sequencing is required for adequate primer design. This can constitute a gargantuan task when addressing heterogeneous groups like coliphages that don't share common DNA sequences. In general, their dependency on skilled personnel, the requirement on costly equipment, together with the inability to differentiate infective from non-infective particles constitute major drawbacks of these methods.

Table 1. Performance of different DNA-based phage detection and quantification methods currently available.

DNA-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
PCR	*Lactobacillus* phages φA2 and φAT3	fermented milk, cheese and whey	centrifugation and DNA extraction	150 min	10^4-10^6 pfu/mL	90 min	Binetti et al. 2008
PCR	*Lactococcus* phage 936-species	whey	concentration-magnetic capture hybridization	300 min	10^2-10^3 pfu/mL	200 min	DuPont et al. 2005
PCR	*Streptococcus* phages	whey	DNA extraction	120 min	10^3 pfu/mL	90 min	Brussow et al. 1994
PCR	*Lactobacillus* phages	whey	DNA extraction and reamplification step	> 150 min	10^2-10^3 pfu/mL	> 180 min	Zago et al. 2006, Zago et al. 2008
PCR	prophages of 30 *Lactobacillus casei* strains	synthetic	MmC induction and DNA extraction	> 20 h	NA	175 min	Zaburlin et al. 2017
Multiplex PCR	*Lactococcus* phages 936, c2, and P335	whey	concentration by 30% PEG	150 min	10^3-10^5 pfu/mL	120 min	Labrie & Moineau 2000
Multiplex PCR	*Lactococcus* phages P335, 936 and c2	milk	no	no	10^3-10^4 pfu/mL	> 120 min	del Rio et al. 2007
qPCR	*Lactococcus* phages 936 and c2	air	air sampling and elution	> 60 min	10^3 genomes/m^3	45 min	Verreault et al. 2011
qPCR	13 *Lactobacillus* phages	milk	no	no	10^5 pfu/mL	30 min	Martin et al. 2008
qPCR	coliphage λ	crude lysate	no	no	< 10^1 pfu/reaction	> 60 min	Edelman & Barletta 2003
qPCR	Ma-LMM01-type cyanophages	pond water	ultracentrifugation and DNA extraction	> 180 min	< 10^3 gc/reaction	NA	Kimura-Sakai et al. 2015
qPCR	halophage SNJ1	underground brine and salt lake water	no	no	10^3 pfu/mL	80 min	Mei et al. 2016

qPCR	STX-converting bacteriophages (Stx phages)	urban sewage, animal wastewater, feces, beef and salad	ultrafiltration and DNA extraction	> 120 min	$< 10^1$ gc/mL $< 10^1$ gc/g	60 min	Imamovic et al. 2010, Imamovic & Muniesa 2011
qPCR	lactophages, c2, 936, and P335	whey and milk	DNA extraction	90 min	10^2 pfu/mL	30 min	Ly-Chatain et al. 2011
qPCR	coliphage M13 and T7	phage display samples	DNase pre-treatment and DNA heat extraction	30 min	10^1 gc/µL	60 min	Peng et al. 2018
qPCR	coliphages MS2 and T4	air	air sampling	0-240 min	future studies	80 min	Usachev et al. 2012
qPCR	*M. aeruginosa* phage	lake water	filtration, concentration and DNA extraction	> 12 h	10^1 gc/mL	60 min	Rozon & Short 2013
qPCR	10 closely related lysogenic lamboid phages	synthetic	MmC induction, DNase treatment and DNA heat extraction	330 min	10^1 gc/reaction	NA	Refardt 2012
qPCR	bacteriophage cocktail 2 (BFC2)	synthetic	no	no	10^3-10^4 gc/mL	< 180 min	Duyvejonck et al. 2019
PMA-long amplicon RT-qPCR	coliphage MS2	synthetic	PMA pre-treatment and RNA extraction	110 min	10^3 pfu/mL	< 90 min	McLellan et al. 2016
Nested RT-qPCR	coliphage MS2	aerosol	air sampling and RNA extraction	> 90 min	10^0 pfu/reaction	170 min	Perrott et al. 2009
Multiplex qPCR	cos and pac-type *S. thermophilus* phages	milk and yoghurt	no	no	10^3-10^4 pfu/mL	120 min	del Rio et al. 2008
Multiplex qPCR	*Campylobacter* phages	chicken meat	concentration and DNA extraction	30-120 min	10^0-10^2 pfu/ml	30 min	Jackel et al. 2017
Integrated cell culture RT-qPCR	F^+-specific RNA genogroup II	shellfish flesh	culture enrichment, centrifugation and RNA extraction	6 h	5 pfu/100g	100 min	Hartard et al. 2017
RT-PCR	F^+-specific RNA genogroups I,II,III,IV	synthetic	RNA heat extraction	10 min	10^0 pfu/reaction	210 min	Friedman et al. 2009

RT-qPCR	F^+-specific RNA genogroups I,II,III,IV	urban raw wastewater	RNA extraction	90 min	10^0-10^1 pfu/mL	150 min	Ogorzaly & Gantzer 2006
RT-qPCR	F^+-specific RNA genogroups I,II,III,IV	faeces and water samples isolates	RNA heat extraction	5 min	1 pfu/g, 10 pfu/100mL	100 min	Ogorzaly et al. 2009, Gourmelon et al. 2010
RT-qPCR	coliphage MS2	synthetic	RNA extraction	90 min	10^2 gc/reaction	95 min	O'Connell et al. 2006
RT-qPCR	F^+-specific RNA genogroups I,II,III,IV	environmental isolates (synthetic)	RNA heat extraction	10 min	10^0-10^2 pfu/reaction	80 min	Friedman et al. 2011
Multiplex RT-qPCR	F^+-specific RNA genogroups I,II,III,IV	seawater and chicken stool	purification and RNA extraction	90 min	10^0 pfu/reaction	180 min	Kirs & Smith 2007
Multiplex RT-qPCR	F^+-specific RNA genogroups I,IV	fecal matter	purification and RNA extraction	90 min	10^3 pfu/g	125 min	Jones et al. 2009
Multiplex RT-qPCR	F^+-specific RNA genogroups I,II,III,IV	shellfish and river water	PEG concentration and RNA extraction	> 10 h	$< 10^1$ gc/reaction	95 min	Wolf et al. 2008
High-throughput qPCR	*Lactococcus* and *Leuconostoc* phages	dairy samples	precipitation, DNase treatment and DNA extraction	> 12 h	10^4 pfu/mL	110 min	Muhammed et al. 2017
Polony method	T7 like cyanophage	seawater	concentration	70 min	10^2 pfu/mL	> 180 min	Baran et al. 2018
ddPCR	*P. syringae* phages FRS and SHL	synthetic and tomato	DNase and Proteinase K treatments	90 min	200 gc/mL	120 min	Morella et al. 2018
gLAMP	coliphages ΦX174, MS2, and PRD1	lake, pond and wastewater	no	no	1 pfu/reaction	25 min	Huang et al. 2018
PCR microchip	λ-DNA template	synthetic	no	no	5 fg/µL	5 min	Hataoka et al. 2005
Disposable PCR Chip	λ-DNA template	synthetic	no	no	200 gc/reaction	60 min	Hataoka et al. 2005
PCR microchip	coliphage MS2	synthetic	no	no	10^4 virions/mL	60 min	Belgrader et al. 1998
Dot blot hybridization	*S. thermophilus* phages	whey	DNA extraction	120 min	10^7 pfu/mL	>18 h	Brussow et al. 1994

1.4.2 Immunoassays

Classical immunoassays such as enzyme-linked immunosorbent assay (ELISA) are techniques based on antigen/antibody reactions in which the antibody is conjugated to a reporter enzyme. The most sensitive type of ELISA uses two antibodies in a sandwich format (Khan et al. 2015). One of the antibodies, fixed to the transducer surface, is used to capture the target. The second antibody, usually linked to an enzyme, is used to provide an amplified signal that can be easily detected through optical or electrochemical methods. Sandwich ELISA is 2 to 5 times more sensitive than indirect or direct ELISA assays that uses only one antibody to detect the antigen. However, it is still 100-1000 times less sensitive than qPCR (Peng et al. 2018). Immunoassays are widely used in clinical diagnostics (Van Emon et al. 2007) due to their practical, easy to operate methodology and high throughput capacity. In these assays phages are used as a safe alternative to more dangerous eukaryotic viruses during method development. For instance, phage MS2 has a shape, size and type of nucleic acid (RNA) similar to waterborne viruses such as enteroviruses, caliciviruses, or hepatitis A (Cho et al. 2005, Debartolomeis and Cabelli 1991), while T7 is a good model for adenoviruses (Kannan et al. 2014). Immunoassays like ELISA have a sensitivity around 10^6 pfu/mL and provide results within 5 hours. To improve the sensitivity by 2 or 3 orders of magnitude, chemiluminescent or fluorescent substrates can be used instead of colorimetric ones as shown in Table 2. This technology has been transferred to a paper-based format, in which phages are immunocaptured. In one case, filter disks were first dipped in primary antibody, then in sample with phages, later in secondary labelled antibody and so on actually mimicking sandwich ELISA technique on a paper (Khan et al. 2015). In other case, filter disks were used as part of a filtration process in which phages where electrostatically trapped during filtration and later on labelled with an antibody enzyme conjugate (Larsson et al. 2013). In both cases, the quantification of phages was performed with a flat-bed scanner and image software in which the color intensity of the product was recorded and analyzed. In addition, semi-quantitative measurements were possible with the naked eye. Paper-based ELISA has a limit of detection of 10^3 pfu/mL and a detection time of 1.5 h (Khan et al. 2015). The filtration-based method is able to detect $5x10^4$ pfu/mL, when the sample volume is increased

to 100 mL (Larsson et al. 2013). Paper based commercial lateral flow assays (LFA), for influenza virus detection (QuickNavi Flu), have detection limits around 10^6-10^7 pfu/mL. Despite their low sensitivity, these assays are relatively cheap, fast and simple to use making them well suited for qualitative applications. However, their quantitative use is not straightforward. An example of a quantitative LFA has been demonstrated when phages are fluorescently labelled with fluorM13 reporter, a genetically biotinylated reporter (immunophage), and quantified by fluorescence microscopy (Kim et al. 2015). The assay provides a sensitivity of 10^4 pfu/mL in approximately 1.5 h. The authors suggest that their LFA can be coupled to a smartphone imaging device, making the method portable and cost affordable. In general, from the sensitivity point of view, immunoassays are inferior when compared to gold standards such as plate-based methods, thus constituting a poor alternative for the detection of low phage concentrations.

Table 2. Performance of different immunoassays for phage detection and quantification currently available.

Immunoassays	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
ELISA	coliphage MS2	synthetic	no	no	$5x10^8$ pfu/mL	300 min	McBride et al. 2003
ELISA	filamentous phage	synthetic	no	no	10^6 pfu/mL	300 min	Shukla & Krag 2005
ELISA	coliphage M13	synthetic	no	no	$4x10^7$ pfu/mL	360 min	Liu et al. 2003
ELISA	coliphage M13	synthetic	no	no	10^7 pfu/mL	300 min	Larrsson et al. 2013
Chemiluminescence ELISA	filamentous phage	synthetic	no	no	10^4 pfu/mL	150 min	Shukla & Krag 2005
Chemiluminescence ELISA	coliphage M13	synthetic	no	no	10^2 pfu/mL	< 8 h	Guttikonda et al. 2004
Enzyme amplified ELISA	coliphage M13	synthetic	no	no	$4x10^4$ pfu/mL	300 min	Liu et al. 2003
Multianalyte immunosensor	coliphage MS2	synthetic	no	no	10^8 pfu/mL	15 min	Taitt et al. 2002
Porous silicon immunosensor	coliphage MS2	synthetic	fluorophore labelling	not specified	$2x10^7$ pfu/mL	60 min	Rossi et al. 2007
Filtration based immunoassay	coliphage M13	synthetic	filtration	< 60 min	$5x10^4$ pfu/mL	60 min	Larsson et al. 2013
Paper-based ELISA	coliphage T7	synthetic	no	no	10^3 pfu/mL	150 min	Khan et al. 2015
LFA-colloidal gold-probe	coliphage M13	synthetic	concentration-two phase micellar system	18 h	$5x10^7$ pfu/mL	30 min	Mashayekhi et al. 2010
LFA-fluorM13 probe	coliphage MS2	synthetic	no	no	10^4 pfu/mL	60 min	Kim et al. 2015
Protein microarray	coliphage MS2	synthetic	no	no	$5x10^7$ pfu/mL	60 min	Rao et al. 2004

1.4.3 Electrochemical-based methods

Electrochemistry is a rather popular approach when designing low-cost and sensitive methods for bacteriophage detection. Techniques can be classified according to the type of electrochemical measurement that happens at the sensor-sample interface, like amperometry and voltammetry. In systems in which direct electron exchange is not possible, redox mediators are used, small molecules that are able to exchange electrons between the sensor and the enzyme of choice. Within this group of methods, it is important to address that the type of electrical measurement is not crucial for the sensitivity, but rather how the system is designed. In the low end of the sensitivity range, we find fluidic methods that rely on amperometric measurements, a type of measurement which measures the relationship between analyte concentration and current at a fixed potential. These methods have a sensitivity of 10^9 pfu/mL within a 1 h time frame (Thomas et al. 2004, Bange et al. 2007). In principle, the assessment of phages was performed in a fluidic system in which the paramagnetic properties of phage specific antibody coated beads were used for the capture and retention of phages at the sensor surface during measurements. Based on these principles a fully automatized phage detection system was developed (Kuramitz et al. 2006). The sensitivity slightly increased when combined in a DNA (Gabig-Ciminska et al. 2004) or immuno chip format (Los et al. 2005) with principles similar to those mentioned above. Unlike amperometry, where measurements are performed at a fixed potential, in voltammetry a changing potential is applied to the working electrode in which the relationship between analyte and current is measured. Voltammetry-based methods are several orders of magnitude more sensitive than amperometric methods with a limit of detection of 10^3 pfu/mL, as shown in Table 3. In this sensitivity range, two rapid methods based on immobilization of antibodies on nanowires made of polypyrrole (Ppy) (Shirale et al. 2010) or carbon nanotubes (CNT) (Garcia-Aljaro et al. 2010) have been developed in a miniaturized chip format. Sensitivity has been improved using a sandwich-based immuno principle on the sensor surface (Prieto-Simon et al. 2015, Prieto-Simon et al. 2014) proving to be a robust and sensitive option when dealing with real water samples. Certainly, these methods are not as rapid as their counterparts due to the sandwich principle of stacking antibodies, which extends

the duration of the assay by 3 h. As a part of their assays, new functionalization antibody strategies for oriented immobilization on building blocks such as carbon nanotubes (Prieto-Simon et al. 2015) and self-assembled monolayers (Prieto-Simon et al. 2014) were also revealed. On the other hand impedance measurements, though not as simple as the previous two methods, measure the resistive and capacitive properties of a material by applying a sinusoidal AC or DC excitation signal at different frequencies (Instruments G 2007). Separate methods have demonstrated low detection limits of 10^2 pfu/mL and even less, but at the expense of increasing the length of the measurements. The most time-consuming, a 6 h method, uses a full-grown biofilm on the sensor surface in which changes in impedance occur upon lysis of the biofilm when exposed to bacteriophages in milk samples (Garcia-Aljaro et al. 2009). By modifying the sensor surface with nanopores, the length of the assay was reduced by 5 h. In this case, an aluminum electrode was anodized and etched to form nanopores of a certain diameter which were then packed with anti-phage antibodies (Chaturvedi et al. 2016). The impedance signal is proportional to the amount of phage particles entrapped in the nanopore. In another nanopore format, anti-phage antibodies were attached to the surface of gold discs at the bottom of silicon nanopores (Brodoceanu et al. 2015). Immuno-nanopore methods have been tested in synthetic samples and demonstrated promising detection capacities in a short period of time, however due to the small diameters of the pores it would be interesting to assess how real complex samples interacts with pores without somehow affecting the sensitivity.

Table 3. Performance of different electrochemical-based phage detection and quantification methods currently available.

Electrochemical based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
Amperometric bead-based immunoassay	coliphage MS2	synthetic	no	no	10^{10} particles/mL	60 min	Thomas et al. 2004
Automated fluidic bead-based immunoassay	coliphage MS2	synthetic	no	no	10^{11} particles/mL	60 min	Kuramitz et al. 2006
NanoIDA bead-based immunoassay	coliphage MS2	synthetic	no	no	10^{9} particles/mL	60 min	Bange et al. 2007
Multiplex DNA chip	coliphages λcIb2, P1vir, T4D and prophage λcI857S7	synthetic	DNA extraction and isolation	< 120 min	10^{7} pfu/ml	30-120 min	Gabig-Ciminska et al. 2004
Amperometric immunochip	coliphage λ and M13	synthetic	no	no	$3x10^{7}$- 10^{8} pfu/mL	50 min	Los et al. 2005
CombiMatrix microarray platform	coliphage M13	synthetic	no	no	$4x10^{8}$ pfu/ml	120 min	Dill et al. 2004
On-chip impedimetric biofilm sensor	coliphage ΦX174	milk and synthetic	no	no	10^{2} pfu/mL	360 min	Garcia-Aljaro et al. 2009
Ionic conductivity immunosensor	coliphage MS2	synthetic	no	no	10^{2} pfu/mL	60 min	Chaturvedi et al. 2016
CNT-based immunosensors	coliphage T7	synthetic	no	no	10^{3} pfu/mL	60 min	Garcia Aljaro et al. 2010
CNT sandwich immunosensor	coliphage MS2	river water	no	no	10^{1} pfu/mL	210 min	Prieto-Simon et al. 2015
SAM sandwich immunosensor	coliphage MS2	river water	no	no	$2x10^{2}$ pfu/mL	210 min	Prieto-Simon et al. 2014
Ppy nanowire-based immunosensors	coliphages T7 and MS2	synthetic, lake and urban runoff water	no	no	10^{3} pfu/mL	5 min	Shirale et al. 2010
Imprinted polymer sensor	*E.coli* phage	5x diluted river samples	no	no	10^{1} pfu/mL	50 min	Erturk & Lood 2018
Voltametric porous silicon immunosensor	coliphage MS2	10x diluted reservoir water	no	no	10^{1} pfu/mL	30 min	Reta et al. 2016
Impedimetric porous silicon immunosensor	coliphage MS2	synthetic	no	no	10^{1} pfu/mL	60 min	Brodoceanu et al. 2015

1.4.4 Mass-based methods

The modus operandi of these methods is to detect change of mass on the device surface due to attachment of analyte. The amount of bound analyte at the sensor surface is usually detected by mechanical methods. Methods like quartz microbalances, acoustic wave sensors and cantilevers are examples of mechanical techniques in which changes in mass attached to the sensor affect the frequency of oscillation of the sensor. Within these group, both quartz microbalance and cantilevers have shown the best detection potential with a sensitivity of 10^3 pfu/mL as shown in Table 4. When using quartz crystal microbalance (QCM) methods, two separated methods generated different detection limits. In the less sensitive approach antibodies were used for the capture molecule and the change in mass was proportional to the number of phages captured on the surface of the sensor, which LOD corresponds to 10^6 pfu/mL (Uttenthaler et al. 2001). In the more sensitive method, first the ligands were used to specifically capture the phage and second, by increasing the amplitude of the oscillation caused breaking of the bond between the ligand and the phage, resulting in acoustic emission when phages detaches from the surface (Dultsev et al. 2001). By implementing this strategy, sensitivity is improved by 3 orders of magnitude when compared to less sensitive QCM method. In the cantilever method, immobilized bacteria on the surface of the cantilever were exposed to different concentrations of bacteriophages which upon lysis caused the cantilever to bend due to mass reduction on sensor surface, with a limit of detection 10^3 pfu/mL and a time of detection of 2 h (Mertens et al. 2019). Common characteristics of mass-based methods are their ability to carry out real time monitoring in a label-free approach, providing valuable information in binding and affinity studies of different molecules and cells in a short period of time. The speed of the assay can be rather affected when complex samples are analyzed. In general, non-specific binding to sensor surface, together with poor sensitivity can constitute a real burden for developing on-site and portable devices able to operate outside well-equipped laboratory facilities.

Table 4. Performance of different mass-based phage detection and quantification methods currently available.

Mass-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
Super high frequency resonator	*Azospirillum lipoferum* phage ΦAl-Sp59b	synthetic	no	no	10^6 phages/mL	10 min	Guliy et al. 2017
Electro-acoustic sensor	*A.lipoferum* phage FAI-SR65	synthetic	no	no	10^6 phages/mL	10 min	Guliy et al. 2018
QCM immunosensor	coliphage M13	synthetic	no	no	$5x10^6$ phages/mL	10 min	Uttenthaler et al. 2001
QCM surface detachment	coliphage MS2	synthetic	no	no	10^3 phages/mL	15 min	Dultsev et al. 2001
Love acoustic wave sensor	coliphage M13	synthetic	no	no	10^9 pfu/ml	< 120 min	Tamarin et al. 2003
Cantilever-immobilized host	coliphage T7	synthetic	no	no	10^3 pfu/mL	120 min	Mertens et al. 2019

1.4.5 Optical-based methods

Methods that rely on optical working principle like surface plasmon resonance (SPR) and optical fibers are methods in which the change in film thickness is displayed as a change in the reflected angle when incident light hits the sensor surface. The surface of the sensor can be modified in numerous ways and in the case of SPR, it has been modified either with susceptible host bacteria, antibodies or imprinted polymers in order to capture and quantify specific phages, as shown in Table 5. In all cases similar detection levels of 10^7 pfu/mL were achieved in only 10 min. The limit of detection can be brought down to 10^2 pfu/mL at the expense of increasing the length of the assay when phages are incubated with immobilized bacteria for 2h (Garcia-Aljaro et al. 2008). In general, SPR measurements in complex samples are somehow affected by non-specific binding of compounds to the sensor's surface. Therefore, samples must be diluted or pre-treated to decrease the matrix interference (Shankaran et al. 2007). Besides, SPR methods due to their high cost and low sensitivity aren't promising tools for the detection of low phage concentration. Relative unexplored methods for phage detection are optical fibers such as long-period fiber gratings (LPFGs). In LPFG sensors, optical fibers are coated with anti-phage antibodies and exposed to solutions containing different phage concentrations (Janczuk-Richter et al. 2017). The detection limit in synthetic samples is relatively good $5x10^3$ pfu/mL for a 30 min assay. In a different study, phages were incubated in the presence of a host inside label-free microdroplets and analyzed by interferometry, for changes in refractive index (Yu et al. 2014). The system uses an optofluidic chip capable of generating and incubating droplets inside the device performing large-scale analysis of thousands of droplets. The assay takes 3 h and has a limit of detection of 10^4 pfu/mL. The pros and cons are similar to those mentioned in mass-based sensors. As positive aspects, they are fast, label free and provides valuable information for binding and affinity studies, but analyses are inconvenient in samples of high complexities due to non-specific binding. Also, operation outside a well-equipped lab presents a burden.

Table 5. Performance of different optical-based phage detection and quantification methods currently available.

Optical-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
SPR immobilized host	coliphage ΦX174 and wastewater phages	synthetic and wastewater	centrifugation and filtration	15 min	10^2 pfu/mL	120 min	Garcia-Aljaro et al. 2008
SPR immobilized host	coliphage T4	synthetic	no	no	10^7 pfu/mL	10 min	Xiao et al. 2012
SPR immunosensor	coliphage MS2	sir	no	no	10^7 pfu/L of air	< 2 min	Usachev et al. 2013
SPR imprinted polymers	coliphage MS2	synthetic	no	no	$5x10^6$ pfu/mL	5 min	Altintas et al. 2015
LPFG immunosensor	coliphage T7	synthetic	no	no	$5x10^3$ pfu/mL	30 min	Janczuk-Richter et al. 2017
OFRR immunosensor	coliphage M13	synthetic	no	no	$2x10^3$ pfu/ml	< 60 min	Zhu et al. 2008
Droplet microfluidic chip	coliphage λ	synthetic	no	no	10^4 pfu/mL	240 min	Yu et al. 2014

1.4.6 Spectroscopy-based methods

Spectroscopy investigates and measures the spectra produced when materials interacts with or emit electromagnetic radiation. Spectral measurement devices are referred to as spectrometers. Methods summarized in Table 6 are either based on UV/Vis absorption spectroscopy, fluorescence spectroscopy or Raman spectroscopy. UV/Vis also known as absorption or reflectance spectroscopy requires the use of a spectrophotometer, in order to perform quantitative measurements. It basically measures the attenuation of a light beam after passing through a sample or after reflectance from a sample surface. Gold nanoparticles, due to surface plasmon resonance phenomena are able to absorb and scatter light with extraordinary efficiency. This feature i.e., the change in color intensity of gold nanoparticles has been exploited for almost immediate detection of phages in liquid suspension (Lesniewski et al. 2014). However, this method which uses antibody conjugated gold nanoparticles has a poor limit of detection of 10^{10} pfu/mL. In a similar method but using a different coating, substituting antibodies with chitosan molecules improves the limit of detection 4 order of magnitudes down to 10^{6} pfu/mL, but at the expense of increasing overall assay length to 1 h (Kannan et al. 2014). Fluorescence spectroscopy analyses fluorescence of a certain excited compound in the sample. In the simplest form fluorometric methods label the phage using a DNA intercalating dye and measure the emission intensity with a fluorimeter (Han et al. 2014). The sensitivity achieved using this method is very limited with a limit of detection of 10^{9} pfu/mL. In a more creative approach, phages were electrophoretically entrapped in nanowells and their fluorescent imprint was analyzed via fluorescent microscopy, decreasing 6 order of magnitude the detection limit down to 10^{3} pfu/mL (Han et al. 2014). In a different approach, bacteria and phages were encapsulated in an emulsion of microdroplets containing a fluorescent non-permeable DNA intercalating dye in which signal intensity is proportional to the numbers of cells that have been lysed (Wang and Nitin 2014). The system is setup in a fluidic structure and the detection is carried out by fluorescence microscopy, showing a LOD of 10^{2} pfu/mL and a detection time of 1 h. In the case of Raman spectroscopy, a light source is used to determine vibrational, rotational and other low frequency modes of a molecule, providing a structural fingerprint by which that molecule is identified. When

applied to detection of phages in raw milk sample, pre-treatment steps like fat separation, removal of casein and filtration, must be performed in order to obtain Raman spectra of the examined phages (Tayyarcan et al. 2018). The method proposed displays high sensitivity and is able to detect 10^2 pfu/mL in 1 h, while allowing to discriminate 2 different groups of phages composed of 8 different *Streptococcus thermophilus* phages and 7 *Lactobacillus bulgaricus* phages.

Mass spectrometry is a powerful analytic technique in which chemical species are ionized and their ions are separated based on their mass-to-charge ratio. Matrix-Assisted Laser Desorption Ionization - Time of Flight (MALDI-ToF), probably the most popular representative among the mass spectrometric techniques, was used to make the first phage fingerprint as early as 1998 (Thomas et al. 1998). In a single work 37 *Staphylococcus* phages were analyzed and tested for quantification purposes (Stverakova et al. 2018). However, identification of phages was possible only if high phage titers ($> 10^7$ pfu/mL) were applied in clean samples.

Table 6. Performance of different spectroscopy-based phage detection and quantification methods currently available.

Spectroscopy-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
MALDI-TOF mass spectrometry	37 different *Staphylococcus aureus* phages	synthetic	no	no	10^7 pfu/mL	< 60 min	Stverakova et al. 2018
Raman spectroscopy	*S. thermophilus* and *L. bulgaricus* phages	raw milk	separation (fat and casein), filtration	< 30 min	10^2 pfu/mL	60 min	Tayyarcan et al. 2018
Laser-induced fluorescence (BC-sense machine)	coliphage MS2	solid copper surface	no	no	10^5 pfu/cm^2	< 5 min	Babichenko et al. 2018
Immuno-gold nanoparticles	coliphage T7	synthetic	no	no	10^{10} pfu/mL	< 1 min	Lesniewski et al. 2014
Chitosan gold nanoparticles	coliphage T7	synthetic	no	no	10^6 pfu/mL	< 50 min	Kannan et al. 2014
Fluorescent plate reader	coliphage T7	synthetic	no	no	10^9 pfu/mL	30 min	Han et al. 2014
Fluorescent nanostructured array	coliphage T7	synthetic	no	no	10^3 pfu/mL	60 min	Han et al. 2014
Water-Oil-Water emulsion microdroplets	coliphage T7	synthetic	no	no	10^2 pfu/mL	60 min	Wang & Nitin 2014

1.4.7 Particle counting-based methods

Viral particle counting can be performed with a flow cytometer or a nanoparticle analyzer. Flow cytometry, is a method in which the analyte (cells or particles) flows one at a time through a laser beam in which light scattering values are used for analyte detection, counting or sorting. The analyte is often labelled with fluorescent markers for better enumeration and discrimination. The limit of detection of this method is 10^4 pfu/mL and the results can be obtained within 2 h. Flow cytometry has been used in MS2 and lactophage studies as indicated in Table 7. In dairy studies, two different approaches were tested in order to determine the concentration of prophage (Oliviera et al. 2017) and phage (Michelsen et al. 2007) initially present in the sample. In the case of detection of prophages, the samples have to be chemically treated with mitomycin C (MmC) to cause induction of temperate phages. Once induced, the phages were fluorescently labelled and quantified by flow cytometry (Oliveira et al. 2017). In the second case, phage-infected cells were compared to non-infected cells on cytograms in which phage-infected cells appeared on the cytogram as cells with low-density cell walls in comparison to non-infected cells (Michelsen et al. 2007).

In either way, samples with complex matrices such as milk require a pre-treatment in order to remove fat particles and eukaryotic cells to prevent clogging and to assure normal performance of the equipment (Michelsen et al. 2007). Unlike flow cytometers, nanoparticle analyzers are strictly label-free methods with an analysis time of just 5min or less as shown in Table 7. Nanoparticle analyzers operates according to a principle identical or very similar to the principle of the Coulter counter. In general, it contains two electrodes which are positioned on both sides of a channel and measures changes in impedance when analyte particle suspended in electrolyte solution pass through the channel. The signals recorded can be related to the size of the analyte and correlated with its concentration. Although, the method has a poor limit of detection of 10^7 phage particles per mL, it can be transformed to a microfluidic format capable of detecting phages in blood plasma (Fraikin et al. 2011). It has been suggested that additional pre-treatment steps would possibly increase the sensitivity levels of this method. In all cases, these methods are well suitable for high-throughput analysis of large phage numbers, however the complexity makes them expensive to

operate and maintain which makes them ill-suited for their use outside the laboratory environment, where clean or purified samples are the exception more than the rule.

Table 7. Performance of different particle counting-based phage detection and quantification methods currently available.

Particle counting-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
Flow cytometry	*Lactococcus lactis* phages	skimmed milk	separation (eukaryotic cells and large fat particles)	not specified	10^5 pfu/mL	60 min	Michelsen et al. 2007
Flow cytometry immunoassay	coliphage MS2	synthetic	no	no	$4x10^7$ pfu/mL	< 90 min	McBride et al. 2003
Flow cytometry immunoassay	coliphage MS2	synthetic	no	no	$3.5x10^6$ pfu/mL	< 120 min	Rao et al. 2004
Flow cytometry	*Lactococcus lactis* prophages	synthetic	MmC induction, filtration, centrifugation and PEG precipitation	> 24 h	10^4 pfu/mL	60 min	Oliveira et al. 2017
Flow cytometry	total virus count	seawater	dilutions and staining	10 min	10^4 pfu/mL	30 min	Brussaard 2004, Brussard 2009
Flow cytometry	total virus count	activated sludge	dilution, staining and DNase treatment	30 min	10^4 pfu/mL	30 min	Brown et al. 2015
Flow cytometry	coliphages λ, P1, and T4	synthetic	dilutions and staining	10 min	10^4 pfu/mL	30 min	Dlusskaya et al. 2019
NanoSight Limited (NS) technology	coliphage ECML17, *Listeria monocytogenes* phage List-36 and *Yersinia Pestis* phage YpP-G	synthetic	no	no	10^7 pfu/mL	5 min	Anderson et al. 2011
High-throughput nanoparticle analyzer	coliphage T7	salt solution and blood plasma	centrifugation (blood)	5 min	10^7 particles /mL	< 1 min	Fraikin et al. 2011
Nanoparticle analyzer	coliphage T2	synthetic	no	no	$5x10^7$ pfu/mL	5 min	DeBlois et al. 1977

1.4.8 Cell-based methods

The essence of cell-based methods is, first, to have an active growing cell culture that is susceptible to phage lysis, and second, to exploit a certain trait of that culture in order to quantify the lysis caused by the phage. A good trait is one that provides distinguishable outcomes in the absence and in the presence of cell lysis and can either be common for all bacteria species or specific for a certain organism. Either way, all cell-based methods provide high sensitivities and times of detection under 4 h, as seen in Table 8. Likely, the simplest trait that can be exploited in all phage-host systems and one of the first observable consequences of the effect of viral infection in bacterial cultures is the decrease in optical density. Another common cell trait upon lysis is the release of intracellular adenylate kinase (AK) and adenosine triphosphate (ATP) which has been exploited in the form of a bioluminescent assay based on the use of the firefly luciferase (Guzman Luna et al. 2009). Regarding the applicability of this approach, phage detection would probably get complicated in ATP rich samples such as blood (Gordon 1986) or milk (Zulak et al. 1976). On other hand, cell-based methods can be modified to detect specific traits genuine for the bacterial specie involved in the detection system. One of the most common specie related marker is ß-galactosidase for *E.coli* detection. This enzyme is characteristic of *E.coli* and few similar species (Saqib et al. 2017) and, therefore, has been used to detect phages that infect *E.coli*, known as coliphages. What makes this enzyme suitable for phage detection and quantification is that it is only secreted into the medium after a lysis event (Ijzerman et al. 1993, Stanek and Falkinham 2001). Prior to use, *E.coli* cell cultures must be induced, in most cases with IPTG, to express ß-galactosidase in amounts suitable for a rapid assay test. The activity of the ß-galactosidase in the phage assay must be measured using non-permeable cell membrane substrates. The method has been converted into a liquid and paper-based formats for water quality monitoring. In the paper method ß-gal induction is performed during the assay, in which a cellulose absorbent pad is used for both coliphage growth and colorimetric detection (Rames and McDonald 2019). The method is able to process and analyze samples as large as 10 mL. Similar methods targeting ß-glucuronidase, another enzyme specific for *Escherichia coli*, *Bacteroides* species, and *Clostridium perfringens* (Skar et al. 1988), have been also developed for

water quality monitoring. A variation of this system uses a genetically modified host to take advantage of the overexpressed β-glucuronidase in order to detect coliphages in samples of up to 50mL (Muniesa et al. 2018).

Undoubtedly, cell-based methods are the most appropriate option for detection of low phage numbers, if a cultivable host is available, however some cautions must be taken into consideration when developing a method. The assays must include controls with a phage resistant organism to exclude any interference by toxic elements that could be found in real samples like antibiotics or heavy metals. Second in terms of quantification, samples contain a mix phage population with different latent periods and burst sizes can provide false estimates of the real number of phages which were initially present in the sample, thus relegating the assays to qualitative detection.

Table 8. Performance of different cell-based phage detection and quantification methods currently available.

Cell-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD	detection time	references
Blue Phage (β-glucuronidase)	coliphages SOM3, SOM23 and ΦX174	synthetic, wastewater, river water, sludge and mussels	filtering of real samples	fast	10^1 pfu/mL	150 min	Muniesa et al. 2018
β-Galactosidase	coliphage β2	synthetic	IPTG induction	< 90 min	10^1 pfu/mL	< 210 min	Ijzerman et al. 1993, Stanek & Falkinham 2001
ATP bioluminescence	coliphage ΦX174	synthetic	no	no	10^1 pfu/mL	180 min	Guzman Luna et al. 2009
QuantiPhage (β-galactosidase)	coliphage ΦX174 and MS2	surface water	no	no	10^1 pfu/mL	180 min	Rames & Macdonald 2019
Latex agglutination culture enrichment method	F^+-specific RNA genogroups I,II,III,IV	synthetic	no	no	10^1 pfu/mL	180 min	Love & Sobsey 2007

1.5 Trends

The need of further development in phage detection and quantification methods is closely related to our understanding of the role of phages in different environments as well as to the progress in key fields such as phage therapy, in the clinical sector. Either way, detection methods that are gaining popularity as of late are cheap, user friendly and don't require expensive instrumentation and pretreatment steps in order to deliver a result. Electrochemical, optical and cell-based methods fulfill these criteria and they have already shown limits of detection equal to the reference DAL method and with a time of detection considerably reduced. These technologies offer the possibility to be reduced in size and fully automated which will be of considerably interest for phage screening or phage detection in different public health and industrial sectors as well as in remote environments. Also, the methods which are suitable for high-throughput format will be of considerate importance in fields mentioned above.

1.6 Objectives

The main goal of this thesis is the development of fast, sensitive and low-cost phage detection and quantification methods that can be implemented in various sectors. The methods must be easy to perform by unqualified personnel while bring maximum reliability. Moreover, the method needs to be eligible for further miniaturization and automatization and be well suited for high-throughput applications. In order to achieve these goals, we have used UV/Vis spectrophotometry to develop a time-kill assay that monitors absorbance during lysis and determines simple kinetic parameters that correlate well with the amount of phages initially present in the sample. In order to perform this quantitative time-kill assay in turbid samples, in which absorbance measurements cannot be used, we have developed a fluorescent method variation based on the initial time-kill assay, that monitors resazurin reduction by actively growing cultures. The results of this research are addressed in the two following chapters.

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2. Fast phage detection and quantification: An optical density-based approach

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Abstract

Since 1959 with the proposal of Double Agar Layer (DAL) method for phage detection and quantification, many sophisticated methods have emerged meanwhile. However, many of them are either too complex/expensive or insensitive to replace routine utilization of DAL method in clinical, environmental and industrial environments. For that purpose, we have explored an alternative method for the detection and quantification of bacteriophages that fulfills the criteria of being rapid, simple and inexpensive. In this thesis we have developed a method based on the analysis of optical density kinetics in bacterial cultures exposed to phage-containing samples. Although the decrease in optical density caused by cell lysis was one of the first observable consequences of the effect of viral infection in bacterial cultures, the potential of the method for the assessment of phage abundance has never been fully exploited. In this work we carry out a detailed study of optical density kinetics in phage-infected bacterial cultures, as a function of both, phage abundance and initial concentration of the host organisms. In total, 90 different combinations of bacteria/phage concentrations have been used. The data obtained provide valuable information about sensitivity ranges, duration of the assay, percentages of inhibition and type of lysing behavior for each phage concentration. The method described can detect, as few as 10 phage particles per assay volume after a phage incubation period of 3.5 h. The duration of the assay can be shortened to 45 min at the expense of losing sensitivity and increasing the limit of detection to 10^8 pfu/mL. Despite using non-sophisticated technology, the method described has shown sensitivity and response time comparable to other high-end methods. The simplicity of the technology and of the analytical steps involved, make the system susceptible of miniaturization and automation for high-throughput applications which can be implemented in routine analysis in many environments.

2.1 Introduction

Methods for the detection and quantification of bacteriophages have been available ever since their discovery by Felix d'Herelle in 1917 (d'Herelle 1917). These methods, based on the presence of lysis plaques in lawns of host bacteria growing in a double agar layer (DAL), were described in detail by Mark Adams in 1959 (Adams 1959) and, with the addition of several modifications and improvements (Cornax et al. 1990, Lillehaug 1997, Sambrook and Russel 2001, Kropinski et al. 2009, Cormier and Janes 2014) they have constituted the workhorse of virus quantification until now. Despite the well-established value of the DAL method, the long times required to achieve detection (24 to 48 h), the labor intensive nature of the methodology, and the impossibility to convert it to an automated or semi-automated format for high throughput testing, make the classical DAL method ill-suited to provide a response to the challenges of current clinical, environmental or industrial applications. In the clinical field, for example, the need to assess phage interference in microbiological diagnostic tools, both pathogen detection and antibiotic susceptibility testing (Brown-Jaque et al. 2016) and the growing need to monitor emerging phage therapy technologies (Cairns et al. 2009, Cooper et al. 2011, Xie et al. 2018, Dalmasso et al. 2015, Harada et al. 2018) call for the development of reliable and fast methods for phage detection. In public health, detection of enteric phages has been proposed as an indicator of fecal contamination in water (Armon and Kott 1995, Muniesa et al. 2018). Finally, the availability of fast phage detection methods in the industrial environment, has been sorely missing for many years. Monitoring of phages responsible for the failure of microbe-based industrial processes such as yogurt or cheese production (Garneau and Moineau 2011, Marco et al. 2012, Jones et al. 2000, Nagai 2012, Li et al. 2015), as well as the use of phages in the biocontrol of food pathogenic bacteria or as an aid in the eradication of biofilms (Harada et al. 2018), all require fast, inexpensive and sensitive methods for routine monitoring applications.

The growing interest in phage monitoring in these fields has prompted the development of a new generation of agile and sensitive methods able to overcome the limitations derived from DAL. These methods are based either on the direct detection of viral particles by PCR (del Rio et al.

2007), qPCR (Ly-Chatain et al. 2011, del Rio et al. 2008), Raman spectroscopy (Tayyarcan et al. 2018), immunoassay (Khan et al. 2015, Larsson et al. 2013), MALDI-TOF (Stverakova et al. 2018, Serafim et al. 2017), or on the lysis of the host organism by flow cytometry (Michelsen et al. 2007), fluorescence microscopy (Wang and Nitin 2014), enzyme release (Muniesa et al. 2018, Stanek and Falkinham 2001, Guzman Luna et al. 2009), surface plasmon resonance (SPR) (Garcia-Aljaro et al. 2008, Altintas et al. 2015) or impedance measurements (Garcia-Aljaro et al. 2009). Sophisticated as they are, many of these methods do not match the sensitivity and precision of the DAL method. Moreover, whereas most of them are considerably faster, the complexity and cost of the instrumentation required for the analysis constitute a definitive barrier for their routine implementation in many environments.

With this in mind, we revisit the idea of using optical density measurements as a simple and inexpensive method for the detection and quantification of bacteriophages in all kind of samples, at different levels of sensitivity and in remarkably short times. Although the decrease in optical density caused by cell lysis was one of the first observable consequences of the effect of viral infection of bacterial cultures, the potential of the method for the assessment of phage abundance has never been fully exploited. In this work we carry out a detailed study calibrating optical density kinetics as a function of both, phage abundance and concentration of the host organisms. Our study determines the percentage of growth inhibition from integrated growth curves and correlates this value to the amount of phage initially present in the sample. The results are discussed in the context of their use in the design of simple and sensitive methods for the monitoring of bacteriophages in industrial, clinical or environmental samples.

2.2 Materials and methods

Microorganisms and growth conditions

Escherichia coli DSMZ 613 (DSMZ, Germany) was grown overnight in Luria-Bertani (LB) medium at 37 °C in an incubator shaker (100 rpm). The cultures were centrifuged at 4000 x *g* for 10 min and resuspended in 1 mL of 0.1 M phosphate buffer (PB, pH=7.2). Optical density of the

cell suspensions was measured at 600 nm using a Smartspec Plus spectrophotometer (Bio-rad, California, USA) and diluted to the required concentration using 0.1 M PB. Bacterial concentrations were determined by viable plate counts and expressed as colony forming unit per mL (cfu/mL).

Bacteriophage T4 was kindly provided by Dr. M. Llagostera from the Department of Genetics and Microbiology of the Autonomous University of Barcelona. Phage lysates were prepared following the protocol of Bonilla et al. (Bonilla et al. 2016) using *E. coli* as a host. 100 mL of an *E. coli* culture growing in LB broth supplemented with $CaCl_2$ (1 mM) and $MgCl_2$ (1 mM) were infected with 100 µL of virus suspension. After achieving lysis, the culture was centrifuged at 4000 x *g* for 20 min. The supernatant was filtered through a 0.22 µm membrane cellulose acetate filter (Whatman) and further treated with chloroform to remove lipids. The resulting suspension was concentrated by ultrafiltration using Amicon Ultra-15 centrifuge tubes with a cutout size of 100 kDa. Additional endotoxin removal, prior to sample storage, was done using 1-octanol as described by Szermer-Olearnik and Boratyński (Szermer-Olearnik and Boratyński 2015) followed by membrane dialysis in a Spectra/Por Float-A-Lyzer G2 Dialysis Device with a MWCO of 3.5-5 kDa. The purified product was stored in SM buffer (Bonilla et al. 2016) at 4 °C. Determination of virus concentration was performed by counting plaque forming unit (pfu) using the double layer agar method described by Adams (Adams 1959) . Prior to their use, virus suspensions were diluted in LB to achieve the desired final concentration.

Experimental design

Our objective was to characterize the optical density kinetics of different combinations of phage/bacteria concentrations in order to assess to what extent kinetic measurements could be used as a reliable indicator of the abundance of phage in a certain sample. Therefore, an experiment was designed in which bacterial concentrations ranging from 10^5 to $5x10^8$ cfu/mL were tested in combination with concentrations of T4 phage ranging from 0 to $5x10^8$ pfu/mL.

Overnight cultures of *E. coli* were centrifuged and the pellets resuspended in 0.1 mM PB to achieve a concentration of 10^{10} cfu/mL. The resulting suspensions were subject to serial dilution in such a way that after mixing with the phage in LB medium the desired final concentration was obtained. In a similar way, stock lysates of T4 were serially diluted in LB medium in order to achieve the desired concentrations. For each assay, 160 µL of LB were mixed with 20 µL phage solution, 20 µL of bacteria solution and 20µL of PB in transparent 96-well plates (Thermo Scientific, Massachusetts, USA). The plates were incubated at 37 °C in a Varioskan Flash plate reader (Thermo Scientific, Massachusetts, USA) and OD_{600} was recorded at regular intervals. Samples, controls and blanks were always assayed as triplicates.

Analysis of the experimental data

The experimental design used provides an extensive set of data that has to be further processed in order to carry out a proper interpretation of the results. For each bacteria concentration used we calculated the Start Point of Detection (SPD) as the time required for the different controls (bacteria without phages) to reach the threshold of detectable growth. We arbitrarily defined this threshold as a growth rate of 0.002 OD units per min. For further calculations we also defined the End Point of Detection (EPD) as a time corresponding to SPD + 120 min, thus allocating a 2-hour window for the assay to develop (Fig 3).

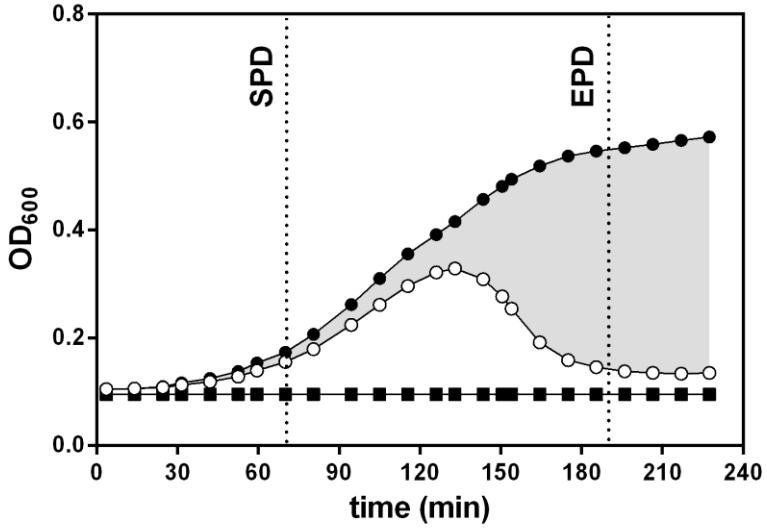

Fig 3. Graphical representation of the procedure used to determine inhibition due to phage lysis in each experiment. Optical density vs time curves of a control (●) and a phage-inoculated culture (○) were integrated and subtracted. The difference, represented by the shaded area, indicates the extent of the inhibition. This area is expressed as a percentage of the area of the control. In cases with little or no phage effect, the shaded area is very small and the percentage of inhibition approaches 0 %. In the most extreme cases the shaded area virtually coincides with the area of the control, and the percentage of inhibition approaches 100 %. In order to standardize all calculations, integration is carried out between the Start Point of Detection (SPD) and End Point of Detection (EPD) as defined in the text.

Growth inhibition due to lysis.

For each bacteria/phage combination, we integrated the area of the curve between the points SPD and EPD. Numerical integration was carried out using the Euler method with the sampling interval as the integration step. The integrated areas were used to calculate a percentage of inhibition (PI) using the following formula based on the procedure described by Xie et al. (Xie et al. 2018):

(Eq. 1) $$PI = \frac{(A_{control} - A_{blank}) - (A_{phage} - A_{blank})}{(A_{control} - A_{blank})} \cdot 100$$

in which $A_{control}$ corresponds to the area of the curve of a control culture without phage inoculation, A_{phage} corresponds to the area of the curve of a culture exposed to a certain phage concentration, and A_{blank} corresponds to the area of the baseline curve consisting only of culture medium without either bacteria or phages (Fig 3). Simplification of Eq. 1 yields:

(Eq. 2) $$PI = \frac{A_{control} - A_{phage}}{A_{control} - A_{blank}} \cdot 100$$

As a rule, in the absence of phage lysis, PI equals 0% and complete lysis gives a PI of 100%. Intermediate results can be correlated to phage concentration for each bacterial concentration used.

Probability of void samples

The probability of void samples (samples containing no phages) was calculated using the probability mass function of the Poisson distribution (Pfeiffer and Schum 1973) expressed as follows:

(Eq. 3) $$P(x = N) = \frac{(c \cdot V)^N \cdot e^{-c \cdot V}}{N!}$$

Where N is the number of phages expected (in this case 0), c is the concentration of phages in the medium subject to sampling and V is the volume of the sample. For the specific case of $N = 0$, Eq. 3 can be simplified to:

(Eq. 4) $$P(x = 0) = e^{-c \cdot V}$$

2.3 Results and discussion

In order to check the suitability of optical density measurements for the detection of low phage concentrations we carried out a series of experiments in which different concentrations of bacteria (10^5, $5x10^5$, 10^6, $5x10^6$, 10^7, $2.5x10^7$, $5x10^7$, 10^8, $2.5x10^8$ and $5x10^8$ cfu/mL) were exposed to different concentrations of phage (0, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$, $5x10^6$, $5x10^7$, and $5x10^8$ pfu/mL). Each combination of phage/bacteria was incubated at 37 °C and optical density at 600 nm was recorded at regular intervals. In total, 90 different combinations of bacteria/phage concentrations were used. Representative results corresponding to three bacterial concentrations (10^8, 10^7 and 10^6 cfu/mL) have been represented in Fig 4. The remaining data can be found in S1 Fig and S1 Dataset. Fig 4A shows the evolution of optical density over time for a 10^8 cfu/mL *E. coli* culture exposed to different initial phage concentrations. As can be seen, optical density of the control increased during the first 90 minutes until the culture reached stationary phase. The effect of phage addition depended to a large extent on the concentration of phage. Addition of $5x10^8$ pfu/mL resulted in a very fast decrease in optical density: after only 25-30 minutes, the culture was completely lysed and optical density had reached the level of the blank. Lower phage concentrations, however, had a less pronounced effect. Thus, 10^7 pfu/mL gave rise to a small decrease in optical density during the first 30 minutes, followed by a second decrease 30 minutes later that brought the culture down to blank levels. This stepwise behavior is highly consistent with the expected kinetics of the lytic cycle for phage T4 which has a latent period of 21 to 35 minutes (Hadas et al. 1997) . With lower phage concentrations (10^6 and 10^5 pfu/mL) cultures grew to some extent before lysis was apparent. Specifically, at 10^6 pfu/mL optical density started to decrease 90 minutes after the beginning of the experiment while at 10^5 pfu/mL this decrease was observed only after 120 minutes of incubation. Below 10^5 pfu/mL ($5x10^4$, $5x10^3$, $5x10^2$ and $5x10^1$ pfu/mL), phage addition had virtually no effect on the kinetics of the culture and the increase in optical density was not much different from that observed in the control.

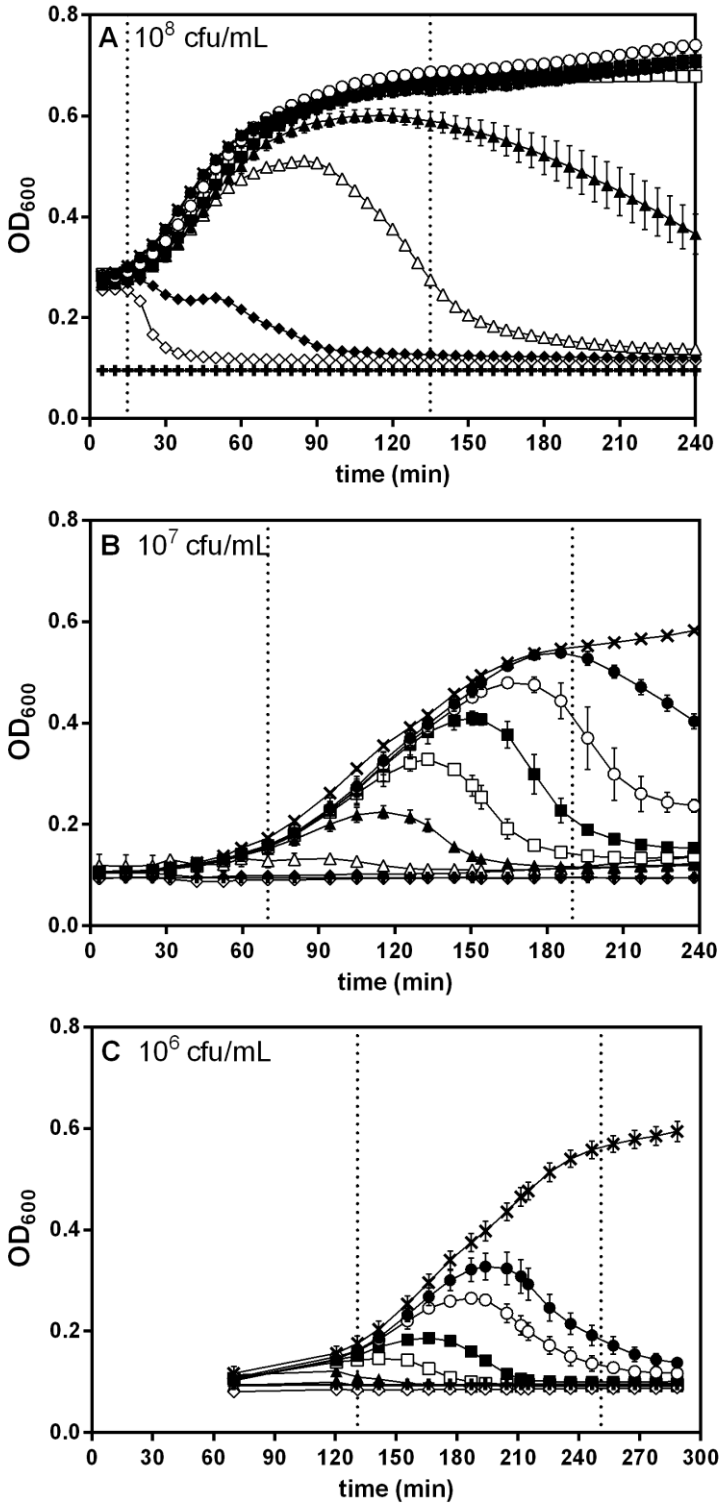

Fig 4. Evolution of optical density during time in cultures of *E.coli* exposed to different T4 phage concentrations. Several bacteria concentrations: **A**) 10^8, **B**) 10^7 and **C**) 10^6 were tested against different phage concentrations: $5x10^8$ (◇), $5x10^7$ (◆), $5x10^6$ (△), $5x10^5$ (▲), $5x10^4$ (□), $5x10^3$ (■), $5x10^2$ (○), $5x10^1$ pfu/ml (●) and without phages (✕). Error bars represent the standard deviation (n=3).

When the same experiment was performed using a tenfold lower cell concentration (10^7 cfu/mL) (Fig 4B) the results were somewhat different. While in the 10^8 cfu/mL culture of Fig 4A optical density started to increase after only 15 minutes of incubation, in this case, OD increase started 70 minutes after the beginning of the experiment. The control without phages showed unrestricted growth which slowed down after 180 minutes. As before, addition of phages had a clear impact on growth dynamics. Even very low amounts of phage ($5x10^1$ pfu/mL) caused detectable cell lysis, with a decrease in OD starting at 190 minutes. Addition of higher phage concentrations shortened the time required for the onset of detectable lysis. That is, the time necessary to detect cell lysis at $5x10^2$, $5x10^3$, $5x10^4$ and $5x10^5$ pfu/mL was progressively shortened from 190 to 133 minutes. A regular trend seems apparent when looking at this data, in which the time required to reach the onset of lysis increased by roughly 20-25 minutes every time that phage concentration was decreased one order of magnitude. Cultures containing phage concentrations above $5x10^5$ pfu/mL did not grow and their OD remained constant over time, indicating that bacterial populations lysed before having the chance to reach detectable OD levels.

Finally, Fig 4C shows the kinetics of OD for a 10^6 cfu/ml *E. coli* culture exposed to the same phage concentrations as above. In this case OD in the cultures only started to increase after 131 minutes. As in the other cases, the control without phages grew unrestricted, but the addition of as little as $5x10^1$ pfu/mL at the beginning of the experiment resulted in the lysis of the culture, with OD starting to decrease at 199 minutes. As before, higher phage concentrations ($5x10^2$ and $5x10^3$ cfu/mL) resulted in lower times to lysis (185 and 166 minutes). Increasing phage concentration above these values resulted in very low or null increases in optical density, once more indicating that the culture had been lysed before having the opportunity to reach a detectable OD level.

Overall, comparison of the three graphs shows several facts: First, decreasing initial cell concentration results in progressively longer lag periods before growth and/or lysis can be detected using optical density. In Fig 4A (10^8 cfu /mL) changes can already be observed 20

minutes after the start of the experiment. When 10^7 cfu/mL are used (Fig 4B) this lag extends to 60 minutes. Use of 10^6 cfu/mL (Fig 4C) further extends this lag to 120 minutes.

In order to assess systematically the magnitude of this delay we recorded the time required for the different controls to reach the threshold of detectable growth. We arbitrarily defined this threshold as a growth rate of 0.002 OD units per min. This time, referred to as the Start Point of Detection (SPD) has been plotted in Fig 5 for all the different conditions used, as a function of initial bacterial concentration. As can be seen in Fig 5, the Start Point of Detection decreases exponentially when increasing cell concentration. Thus, at the lowest cell concentration used (10^5 cells/mL), SPD is 200 minutes. This time decreases at a rate of 70 minutes per log increase in cell concentration, down to approximately 20 minutes. In the same graph, the End Point of Detection (EPD) has also been represented. As explained in the methods section, EPD is calculated as SPD + 120 min and corresponds roughly to the time required to carry out reliable phage detection at each bacterial concentration. EPD values range from a maximum of 5.5 h when using 10^5 cfu/mL, to 2 h 15' when using higher cell concentrations.

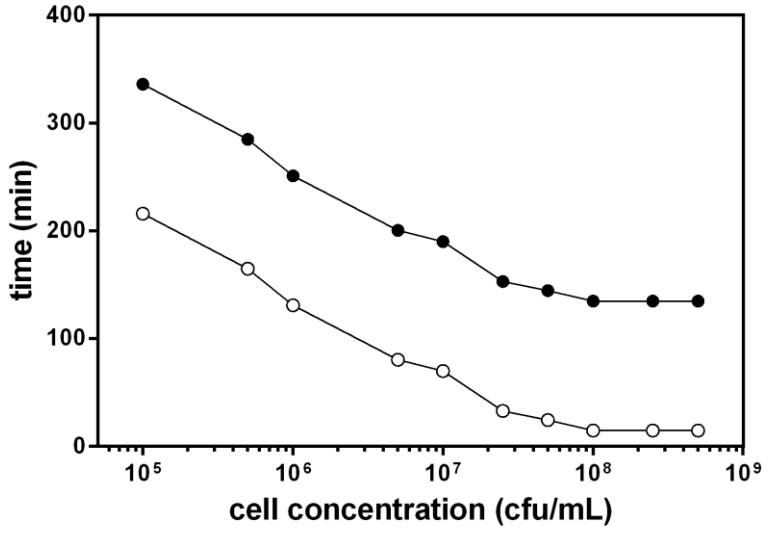

Fig 5. Representation of the Start Point of Detection (SPD) (○) and End Point of Detection (EPD) (●) as a function of the concentration of bacteria used in the experiment. SPD is the time at which detectable growth (defined as ≥ 0.002 OD units per minute) starts. EPD is defined as SPD + 120 minutes, the time usually required to carry out a reliable phage detection.

A second observation concerning the experiment presented in Fig 4 refers to the range of phage concentrations that can be detected using each cell concentration. In general, the kinetics of optical density show three types of behavior:

I) **No lysis. No effect on growth**. High bacterial concentrations combined with low phage concentrations result in unrestricted growth that most of the times cannot be differentiated from the growth kinetics of the control. This can be seen in Fig 4A when 10^8 cfu/mL are exposed to 10^4, 10^3, 10^2 and 10^1 pfu/mL).

II) **Complete lysis. No growth**. Low bacterial concentrations combined with high phage concentrations display no detectable growth as the complete culture is lysed before optical density starts to increase. This behavior can be observed in Fig 4 C, in which 10^6 cfu/mL exposed to 10^6, 10^7 and 10^8 pfu/mL show virtually no growth.

III) **Delayed lysis**. A detectable increase in OD occurs, but after a certain time, which depends on the concentration of phage, OD starts to decrease as a consequence of bacterial lysis. This can be observed in Fig 4B (10^7 cfu/mL) when the culture is exposed to 10^1, 10^2, 10^3, 10^4 and 10^5 pfu/mL.

The type of behavior observed has been recorded for each of the 90 different combinations of phage/bacteria assayed. The results are shown in Fig 6. Data have been encoded in such a way that **Complete Lysis** is represented as a very small dot, **No Lysis** appears as a large size circle, and **Delayed Lysis** is shown as an intermediate sized circle. As can be seen in the right hand side of the graph, cultures with high cell concentrations are not sensitive to low phage numbers as the culture reaches stationary phase before the phage has had time to propagate enough to cause detectable lysis. In opposition, on left hand side of the graph it can be observed how low concentrations of bacteria are completely lysed by phage concentrations of $5x10^4$ pfu/mL or higher. As a rule, decreasing initial cell concentration improves detection at low phage titers, but there is a tradeoff. Use of low cell concentrations, as seen in Fig 5, increases considerably the time required for the assay. In general terms, the best results for phage detection were obtained with the use of $5x10^6$ and 10^7 cfu/mL. In this concentration range, **delayed lysis** was detected for samples containing only 50 pfu/mL with a short incubation between 2 and 3 hours.

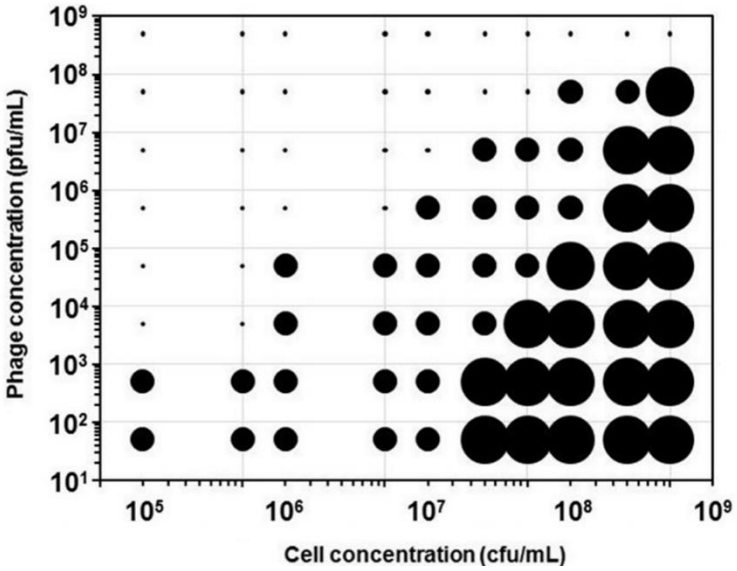

Fig 6. Lysis behavior of the different combinations of T4 and *E. coli* concentrations assayed. Large circles indicate the absence of lysis; small dots indicate complete lysis right from the beginning of the experiment. Intermediate circles indicate the existence of delayed lysis, this is, significant bacterial growth can be observed before the onset of lysis.

In an attempt to make the assay quantitative, we used the procedure described in Materials and Methods to calculate the % inhibition (PI) caused by the presence of phages in each sample. The results, corresponding to each bacterial concentration, are presented in Fig 7 as a function of phage concentration. Fig 7 can be read as a set of calibration curves, each carried out at a different concentration of bacteria. In general, high bacterial concentrations are only sensitive to very high phage concentrations. At the same time, detection of low phage concentrations requires the use of low bacterial concentrations. To exemplify this, the calibration obtained with 10^8 cfu/ mL provides a 3 log dynamic range between $5x10^5$ and $5x10^8$ pfu/mL. In the case of the curve obtained using 10^7 cfu/mL, the dynamic range stretches 4 log between $5x10^2$ and $5x10^6$ pfu/mL. At 10^6 cfu/mL, the sensitivity range narrows again to 3 log but allows detection of much lower phage concentration, between $5x10^1$ and $5x10^4$ pfu/mL.

Each of the points in the graph has been calculated from data of experiment carried out in triplicate. For each set of replicates, variability was always low with relative standard errors averaging 3.5% of the means. In order to see whether this experiments could be consistently reproduced the measurements corresponding to 10^7 cells/mL were repeated three times at

different dates using different inocula and different batches of culture medium and reagents. The results of these experiments allowed the estimation of an independent standard error for the measurements which has been included as a set of error bars for the 10^7 cells/mL curve. In most cases standard errors are between 1 and 3% of the mean and, therefore, error bars are smaller than the symbols used in the graph. In two cases standard errors reach 4% of the mean and can be actually be seen as error bars expanding beyond the symbol. Overall, our conclusion is that the results are highly consistent and can be accurately reproduced in experiments carried out independently.

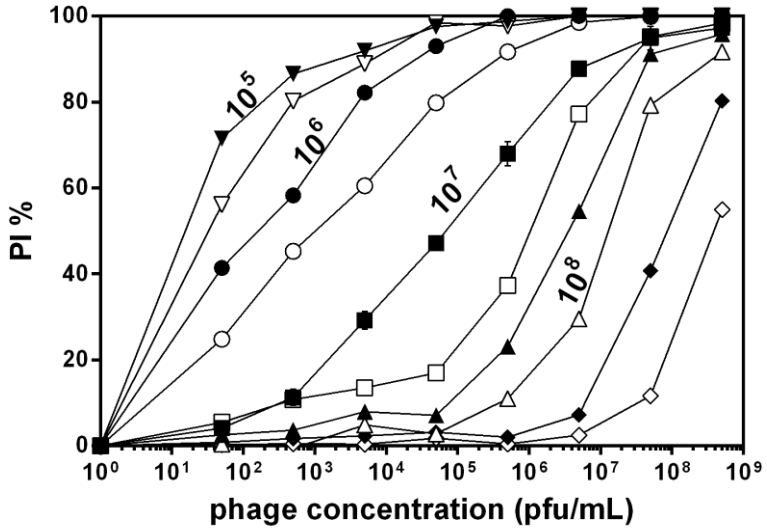

Fig 7. Percentage of inhibition as a function of phage concentration for different values of cell concentration: $5x10^8$ (◇), $2.5x10^8$ (◆), 10^8 (△), $5x10^7$ (▲), $2.5x10^7$ (□), 10^7 (■), $5x10^6$ (○), 10^6 (●), $5x10^5$ (▽), 10^5 cfu/ml (▼). Percentages of inhibition were calculated as described in Materials and Methods.

On the other hand, the detection limit of the method is inherently tied to the small volumes at which the assay is carried out. In a typical microplate assay, a working concentration of 50 pfu/mL (10 phages per microwell) in the microplate well requires taking 20 µL of a 500 pfu/mL sample in a total volume of 200 µL of phage + bacterial culture. The probability under these conditions of having a 20 µL sample containing zero phages, calculated using the probability mass function of the Poisson probability distribution (Material and Methods, Equation 4), is $4.54x10^{-5}$ which means that only one out of approximately 22.000 samples will not contain

phages. Lowering down the concentration to 5 phages per mL in the microplate well would require taking 20 µL of a 50 phage/µL sample in a total assay volume of 200 µL. Under these conditions the probability of having samples with no phages increases considerably, up to 0.368. At this probability practically 1 of every 3 samples would come void decreasing considerably the reliability of the assay.

Therefore, based on the design of the microplate assay and the volumes of sample involved, this method is able to detect 50 phages corresponding to an actual concentration of 500 phages/mL in the original sample. The time required for the assay under this conditions is 3.5 hours at the most, but this time can be reduced considerably when attempting to detect higher phage concentrations. Thus, detection of 10^8 phages/mL can carried out in only 45 minutes.

In order to compare the method described in this chapter with methods previously described in the literature, the performance of currently available methods, using nucleic acid detection, immunoassay, electron microscopy, impedance, SPR or release of intracellular components, has been summarized in Table 9. The sensitivity of these methods ranges across several orders of magnitude. At the low sensitivity end of the spectrum, electron microscopy provides precise quantification in a short time, but it requires high phage titers ($\geq 10^7$ phages/mL) to provide reliable results. In addition, electron microscopy requires expensive equipment and highly skilled personnel, while providing a very low analytical throughput. At the other end, the highest sensitivity is found in methods that measure the release of intracellular components (ATP, ß-galactosidase, ß-glucuronidase) which allow the detection of 10^1 phages/mL with short protocols requiring 2-3 hours of assay. The remaining methods have limits of detection in the 10^2-10^3 phages/mL range with time-to-result between 1 and 6 hours. The assay proposed in this chapter fits in this middle segment. Using relatively simple equipment it is possible to detect 10^2 phages/mL in 3.5 hours, a time that can be shortened considerably at the expense of increasing the limit of detection.

In this chapter we do not describe a fully applicable method. The results obtained with our *E. coli*/T4 model system cannot be directly extrapolated to other bacteria/phage systems. But we establish a proof of concept that shows that kinetic-based methods can provide reliable phage

detection and quantification in a reasonably short period of time. The chapter also describes a methodology backed up by a very extensive data set, that can be used as a solid framework for the development of solutions to specific problems. Development of methods for the detection of phage levels in food preservation applications or in phage therapy, or detection of phages in industrial or environmental applications would demand an extensive full-fledged study requiring careful standardization, a characterization of the effect of the analytical matrix and taking into account the kinetics of the particular phage/host system that was beyond the scope of our work. The approach we propose is not devoid of problems. Samples containing toxic compounds might show inhibition in the absence of phages thus leading to false positive readings. Also, the samples could contain heterogeneous phage populations with very different infection kinetics, thus precluding accurate calibration and quantitative use, relegating the assay to a qualitative detection method. The effect of toxicity can be addressed, if required, by separately assessing toxicity or including phage-resistant organisms as controls. All of this elements, as mentioned above, are part of a specific method development and should be taken into account for each specific application.

Table 9. Sensitivity, expressed as the limit of detection, and time required for detection, in different methods currently available for phage detection.

Methods	Limit of detection (pfu/mL)	Time to detection (h)	Reference
OD kinetics	10^2	0.75-3.5	this work
Surface Plasmon Resonance (SPR)	10^2	3	(Garcia-Aljaro et al. 2008)
Impendance measurements	10^2	6	(Garcia-Aljaro et al. 2009)
ß-glucuronidase release	10^1	2.5	(Muniesa et al. 2018)
ß-galactosidase release	10^1	2.5	(Stanek & Falkinham 2001)
ATP release	10^1	3	(Guzman Luna et al. 2009)
DNA - qPCR	10^2	2	(Ly-Chatian et al. 2011)
DNA - qLAMP	10^3	1	(Huang et al. 2018)
DNA - PCR	10^3	4	(del Rio et al. 2007)
Antibodies - Paper based ELISA	10^3	2	(Khan et al. 2015)
Antibodies - Carbon nanotubes	10^3	1	(Garcia-Aljaro et al. 2010)
Fluorescence microscopy	10^2	1	(Wang & Nitin 2014)
Transmission electron microscopy	10^7	1	(Blancett et al. 2017)

In summary, this study presents a model based on the measurement of OD kinetics for phage enumeration and detection, using simple and inexpensive equipment. Although it uses non-sophisticated technology it has shown sensitivity and response time comparable to other high-end methods. Due to the simplicity of the technology and of the analytical steps involved, we anticipate that the system is susceptible of miniaturization and automation for high-throughput applications.

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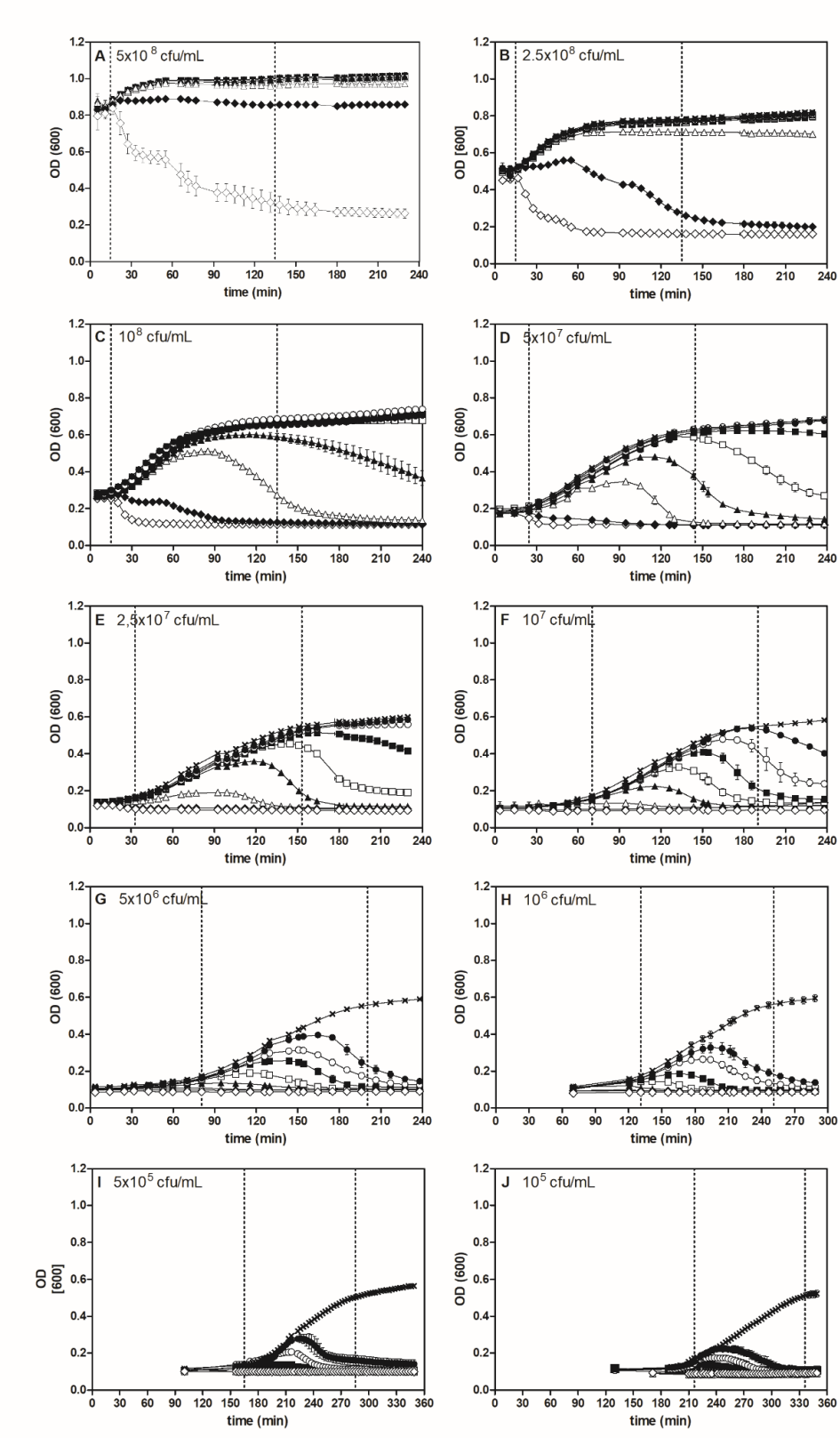
2.5 Supporting information



S1 Fig. Evolution of optical density during time in cultures of *E.coli* exposed to different T4 phage concentrations. Several bacteria concentrations: **A**) $5x10^8$, **B**) $2.5,10^8$, **C**) 10^8, **D**) $5x10^7$, **E**) $2.5x10^7$ **F**) 10^7 **G**) $5x10^6$, **H**) 10^6, **I**) $5x10^5$ and **J**) 10^5 were tested against different phage concentrations: $5x10^8$ (◇), $5x10^7$ (◆), $5x10^6$ (△), $5x10^5$ (▲), $5x10^4$ (□), $5x10^3$ (■), $5x10^2$ (○), $5x10^1$ pfu/ml (●) and without phages (×). Error bars represent the standard deviation (n=3).

S1 Dataset contains the optical density (OD) vs time data corresponding to 90 different phage/bacteria combinations. Data have been used to build the graphs in Supplemental Fig S1. A subset of the data has been used for Fig 4. The complete dataset can be found in 5.1.1 Annex.

3. Fluorometric detection of phages in liquid media: Application to turbid samples

This work has been published in the journal Analytica Chimica Acta (5.2 Annex)

Abstract

During the last years there has been a growing interest in the development of methods for phage detection and quantification in environmental, public health and industrial sectors. Good methods of phage monitoring contribute to progress in phage therapies, biocontrol and food safety studies. They have also been used to indicate the possible presence of microbiological hazards in drinking and recreational waters, and are an essential tool to prevent failure of microbe-based industrial bioreactors. Many of the sophisticated methods that have emerged to cover these needs are strongly hampered by the presence of turbidity in the samples, that results in decreased sensitivity. To avoid this, time consuming pretreatment steps must often be included that increase the overall complexity of the assays and the time required to perform them. With this in mind, we have explored an alternative method that fulfills the criteria of being simple, rapid and inexpensive and can be used to perform analysis in turbid media without any pretreatment steps. In this chapter we develop a method that monitors lysis of an indicator culture when exposed to samples containing the target phage. The method is based on the properties of resazurin, a redox dye that becomes fluorescent when reduced by an active microbial culture. We analyzed the fluorescence kinetics of non-turbid phage-infected bacterial cultures as a function of both, phage abundance and initial cell concentration. For this purpose, different phage/host combinations were used and then, the addition of resazurin at different times (0, 30 and 60 minutes) was carefully evaluated for each phage/host combination, thus providing data for 186 combinations in total. Next, selected phage/host combinations were tested over 4 different turbidity models: 0, 1000, 2000 Nephelometric Turbidity Units (NTU) as well as in milk. The data obtained provided information about the duration of the assay and sensitivity thresholds in matrices with different turbidity grades. The results obtained indicate that the method can detect as few as 10 phage particles per assay volume within 3.5 h. If sensitivity is not an issue and the threshold of detection is increased to 10^7 phages the assay is considerably shortened, providing reliable results in only 40 minutes.

Overall, the detection approach proposed in this work provides a simple, rapid and inexpensive solution that compares favorably, in terms of performance, with other high-end methods.

3.1 Introduction

During years, interest in phage detection and quantification has been mainly restricted to the academic world. However, the growing understanding of how bacterial viruses impact applied processes, together with the emergence of new applications that use phages as tools for environmental diagnostics, clinical therapy or food safety (Harada et al. 2018, Mutti and Corsini 2019, Muniesa et al. 2018), have given rise to the need for methods of phage detection that are both, fast and reliable, and liable to automatization (Cooper et al. 2011, Xie et al. 2018, Storms et al. 2019). In the context of phage therapy, the importance of rapid monitoring of phages in samples such as blood, sputum or biofilms is necessary for the successful implementation of these treatments (Mutti and Corsini 2019 Hodyra-Stefaniak 2015, Cairns et al. 2009, Dalmasso et al. 2015, Milho et al. 2019). Also, according to water and food safety and quality guidelines the presence of certain phages in drinking and recreational waters (Muniesa et al. 2018, Armon and Kott 1995, Rames and Macdonald 2019), as well as in shellfish meat lysates (Wolf et al. 2008) can be related to the existence of faecal contamination and, therefore, be used as a tool for risk management. In industrial environments, monitoring of phages is important, to prevent failure in microbe-based processes such as cheese, yogurt, acetone production and others (Garneau and Moineau 2011, Marco et al. 2012, Jones et al. 2000, Nagai 2012, Li et al. 2015). Also, the use of phages for biocontrol of food pathogenic bacteria, as well as for the eradication of biofilms in industrial environments (Harada et al. 2018) require sensitive, simple and fast monitoring methods. While the reference Double Agar Layer (DAL) method (Kropinski et al. 2009, Adams 1959) provides very accurate results using basic microbiological techniques, it is too slow for applications requiring quick response. The need for fast and reliable methods of phage detection and quantification has prompted the emergence of a number of methods relying on techniques such as, transmission electron microscopy (TEM) (Zhang et al. 2013), surface plasmon resonance (SPR) (Garcia-Aljaro et al. 2008), immunoassay (Khan et al. 2015), qPCR (Martin et al. 2008,

Ly-Chatain et al. 2011, Jackel et al. 2017), MALDI-TOF (Stverakova et al. 2018), Raman spectroscopy (Tayyarcan et al. 2018), enzyme (Muniesa et al. 2018, Ijzerman et al. 1993) or ATP release (Guzman Luna et al. 2009), flow cytometry (Oliveira et al. 2017), OD kinetics (Rajnovic et al. 2019), impendence (Garcia-Aljaro et al. 2009) or capacitive measurements (Erturk and Lood 2018). In some of the methods mentioned above, phage detection can be hampered by the presence of turbidity in the sample. Dealing with this turbidity requires the application of pre-treatment steps such as extraction, dilution, purification or filtration to eliminate this interference. Incorporation of these treatments increases the complexity, the length and the cost of the assays often requiring participation of qualified personnel. As a rule, incorporation of pre-treatment steps constitutes a definite barrier for their routine implementation for many applications.

In order to develop a method that can detect phages in turbid samples without the need of a pre-treatment, and that can be adapted for high-throughput analysis, we have opted for monitoring phage-induced changes in microbial activity through the use of resazurin. In the presence of metabolically active cultures resazurin is reduced to fluorescent resorufin in such a way that this reduction can be used to assess microbial activity levels (Balouiri et al. 2016). We have exploited this property to detect and quantify phages in samples with different turbidities. As a turbidity model we have used calibrated polymer bead suspensions of different Nephelometric Turbidity Units (NTU). As an additional control we have included milk as an example of a natural turbid sample that can be analyzed using this approach, mainly to show its validity in a more complex analytical matrix

The results are discussed in the context of designing a simple and inexpensive procedure for the detection of low phage numbers in turbid samples, as often found in public health, industrial and environmental studies.

3.2 Materials and methods

Microorganisms and growth conditions

Escherichia coli DSMZ 613 (DSMZ, Germany) was grown overnight in Luria-Bertani (LB) broth at 37 °C in an orbital incubator (Infors HT Ecotron, Switzerland) at 100 rpm. Once grown, the cultures were centrifuged 10 min at 4000 x *g* and resuspended in 1 mL of 0.1 M phosphate buffer (PB, pH=7.2). Optical density was measured at 600 nm using a Smartspec Plus spectrophotometer (Bio-rad, California, USA) and further diluted to the desired concentration using 0.1 M PB. Viable plate counts using LB plates were used to determine bacterial concentrations. The results have been expressed as colony forming unit per mL (cfu/mL).

Bacteriophage T4 was used throughout the work and was kindly provided by Dr. M. Llagostera from the Department of Genetics and Microbiology of the Autonomous University of Barcelona. Phage lysates were prepared as described by Bonilla et al. (Bonilla et al. 2016) using *E. coli* as a host organism. 100 mL of LB medium supplemented with $CaCl_2$ (1 mM) and $MgCl_2$ (1 mM) were inoculated with 10 mL of an *E. coli* overnight culture and later infected with 100 µL of virus stock suspension. After achieving complete lysis, the culture was centrifuged 20 min at 4000 x *g*. The supernatant was filtered through a 0.22 µm cellulose acetate membrane filter (Whatman) and treated with chloroform to remove lipids. The supernatant was concentrated by ultrafiltration using Amicon Ultra-15 centrifuge tubes with 100 kDa cutoff size. Prior to sample storage, an additional endotoxin removal protocol (Szermer-Olearnik and Boratynski 2015) was applied by using 1-octanol, followed by membrane dialysis in a Spectra/Por® Float-A-Lyzer® G2 Dialysis Device with a MWCO of 3.5-5 kDa. The obtained purified product was stored at 4 °C in SM buffer (Szermer-Olearnik and Boratynski 2015). The double layer agar method described by Adams (Adams 1959) was used to determine virus concentration, expressed as plaque forming unit (pfu). Prior to their use, virus suspensions were diluted in PB to achieve the required final concentration.

Experimental design

In order to explore to what extent the resazurin reduction provided a clear indication of bacterial lysis, and in an attempt to assess the sensitivity of the method to low phage concentrations, we designed an experimental setup in which 9 different concentrations of T4 phage (0, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$, $5x10^6$, $5x10^7$, $5x10^8$ pfu/mL) were tested in combination with 10 different concentrations of *E. coli* (10^5, $5x10^5$, 10^6, $5x10^6$, 10^7, $2.5x10^7$, $5x10^7$, 10^8, $2.5x10^8$, $5x10^8$ cfu/mL). For each of the combinations we monitored lysis through the kinetics of fluorescence development due to resazurin reduction by the active microbial culture.

In a separate experiment we analyzed the effect of turbidity in the measurements by exposing 10^7 cfu/mL of *E. coli* to different concentrations of phage (0, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$, $5x10^6$, $5x10^7$ pfu/mL) in media of different turbidity (1000, 2000 NTUs and milk). Controls without phage were used throughout the work. In all cases, samples, controls and blanks were assayed as triplicates.

For the experiments, overnight cultures of *E. coli* were centrifuged and resuspended in 0.1 mM PB to obtain a desired stock concentration of 10^{10} cfu/mL. The suspensions were serially diluted in PB and added to the assay mix to provide the final concentration desired. In an identical way, stock lysates of T4 were serially diluted in PB to achieve the desired concentrations. 10mM stock solutions of resazurin (Panreac, Spain) were prepared in PB and added to the samples at a final concentration of 1mM. Furthermore, 4000 NTU latex bead standard (Sigma Aldrich, Germany) and commercial UHT milk were used to make solutions of different turbidities. For each assay, 60 µL of SB (Super Broth, 3x concentrated LB medium, pH=7) were mixed with 20 µL phage solution, 20 µL of bacteria solution and 20 µL of RSZ in transparent 96-well plates (Thermo Scientific, Massachusetts, USA). Depending on the experiment, 100 µL, 50 µL or 0 µL of a 4000 NTU turbidity standard, or 20 µL of milk were added to the well. In all cases, PB was used to bring the assay volume up to 220 µl. The plates were incubated at 37 °C in a Varioskan Flash plate reader (Thermo Scientific, Massachusetts, USA) and relative fluorescent units (RFU) were

recorded at 3 minute intervals using 560 nm as the excitation wavelength and 590 nm as the emission wavelength.

Data analysis

The experimental design used provided an extensive set of data that had to be further processed in order to carry out a proper interpretation of the results. The procedure used to process the data is presented in Fig. 8. For each concentration of bacteria, we calculated the Time of Detection (ToD) as the time required for the control (bacterial culture without phages) to reach the highest RFU value. The lysis threshold limit was arbitrarily defined and characterized as a positive result, indicating the presence of phages, when the sample had half the RFU of the control at ToD. Fluorescent values above this threshold were defined as "no lysis", therefore indicating the absence of phages.

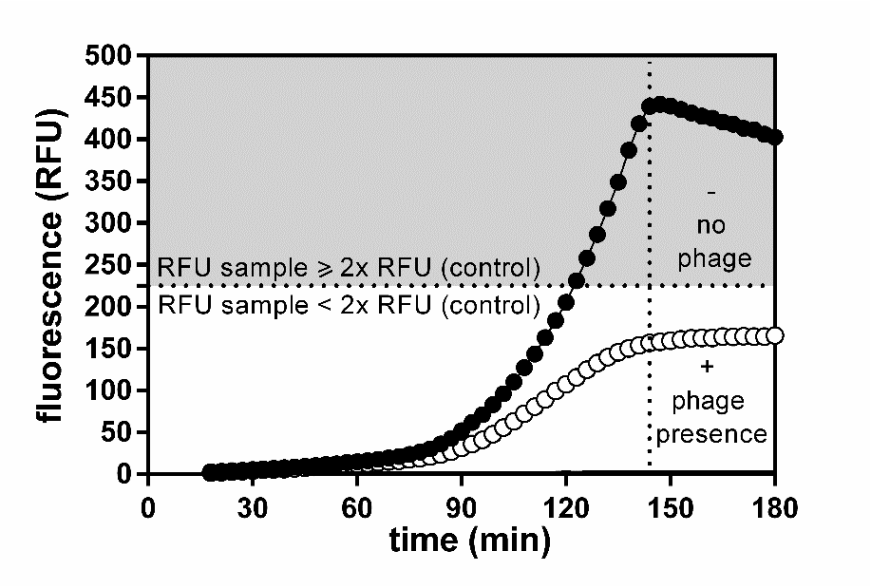


Fig 8. Graphical representation of the procedure used to determine phage threshold limits in each experiment. Fluorescence vs time curves of a control (●) and a phage-inoculated culture (○) of *E. coli*. We calculated the Time of Detection (ToD) (indicated by a vertical dotted line) as the time required for the control (bacterial culture without phages) to reach the highest fluorescence value. The lysis threshold limit was arbitrarily defined as the fluorescence value corresponding to ½ the fluorescence of the control at ToD. Samples above this threshold (shaded area) were considered as negative, indicating the absence of phages. Samples with fluorescence values below this threshold (non-shaded area) where considered as positive, indicating the presence of phages.

The probability of void samples (samples containing zero phages) was calculated as previously described (Rajnovic et al. 2019) using the mass function of the Poisson distribution (Pfeiffer and Schum 1973)

$$P(x = 0) = e^{-c \cdot V} \quad \text{(Eq. 5)}$$

Where c is the concentration of phage in the sample, and V is the volume of sample being analyzed.

3.3 Results and discussion

In order to evaluate the applicability of fluorescent measurements for the detection of low phage concentrations we designed a set of experiments in which 10 different bacterial concentrations (10^5, $5x10^5$, 10^6, $5x10^6$, 10^7, $2.5x10^7$, $5x10^7$, 10^8, $2.5x10^8$ and $5x10^8$ cfu/mL) were exposed to 9 different phage concentrations (0, $5x10^1$, $5x10^2$, $5x10^3$, $5x10^4$, $5x10^5$, $5x10^6$, $5x10^7$, and $5x10^8$ pfu/mL). Each of the 90 phage/bacteria combinations was incubated at 37 °C and after addition of resazurin, fluorescence of the samples was recorded until stable values were reached, a period of 2 to 6 hours depending on the experiment. When designing the experiment, we faced the dilemma of either adding resazurin at the beginning of the incubation, or to allow some time before addition so that phages could start lysing the culture and we could better discriminate between infected or non-infected samples. To assess whether the time of resazurin addition affected the kinetics of fluorescence development, resazurin was added to the bacteria/phage mix after either 0, 30 or 60 minutes of preincubation at 37 °C. The results have been plotted in Fig. 9 for the 10^8 cfu/mL-$5x10^7$ pfu/mL bacteria-phage concentrations. Fig. 9A shows the kinetics of fluorescence increase in controls without phages. As can be seen in this figure, when resazurin was added at time zero, fluorescence increased steadily until reaching a maximum after approximately 60 minutes indicating the Time of Detection (ToD) as defined in Fig. 8. Delaying resazurin addition by 30 minutes caused a delay of only 10 minutes as the higher biomass produced during the 30 minutes preincubation resulted in faster resazurin reduction as shown by the steeper slope. When resazurin was added after 60 minutes of preincubation the slope of the curve was even steeper and as a results ToD was only delayed by 20 minutes. From the point of

view of the ability to discriminate between presence and absence of phages the results were very similar for all preincubation times, showing fluorescence values much lower than the controls (Fig. 9B). Stable fluorescence values for the 0 and 30 minute preincubations were virtually identical. This was probably a consequence of the fact that T4 has a latent period of 21-35 min (Hadas et al. 1997) and the cultures preincubated for 30 min barely had time to lyse before the addition of resazurin. In the case of samples preincubated for 60 minutes, stable fluorescence was lower as the culture had less time to reduce the reagent before lysis was complete. The effect of preincubation was checked for different bacteria-phage concentrations (Supplemental Fig. S2). The results were consistent in all cases, indicating that the existence of a preincubation period before addition of the reagent did not enhance in a significant way the sensitivity of the assay. Therefore, the results shown from now on will refer to experiments in which resazurin was added at time zero.

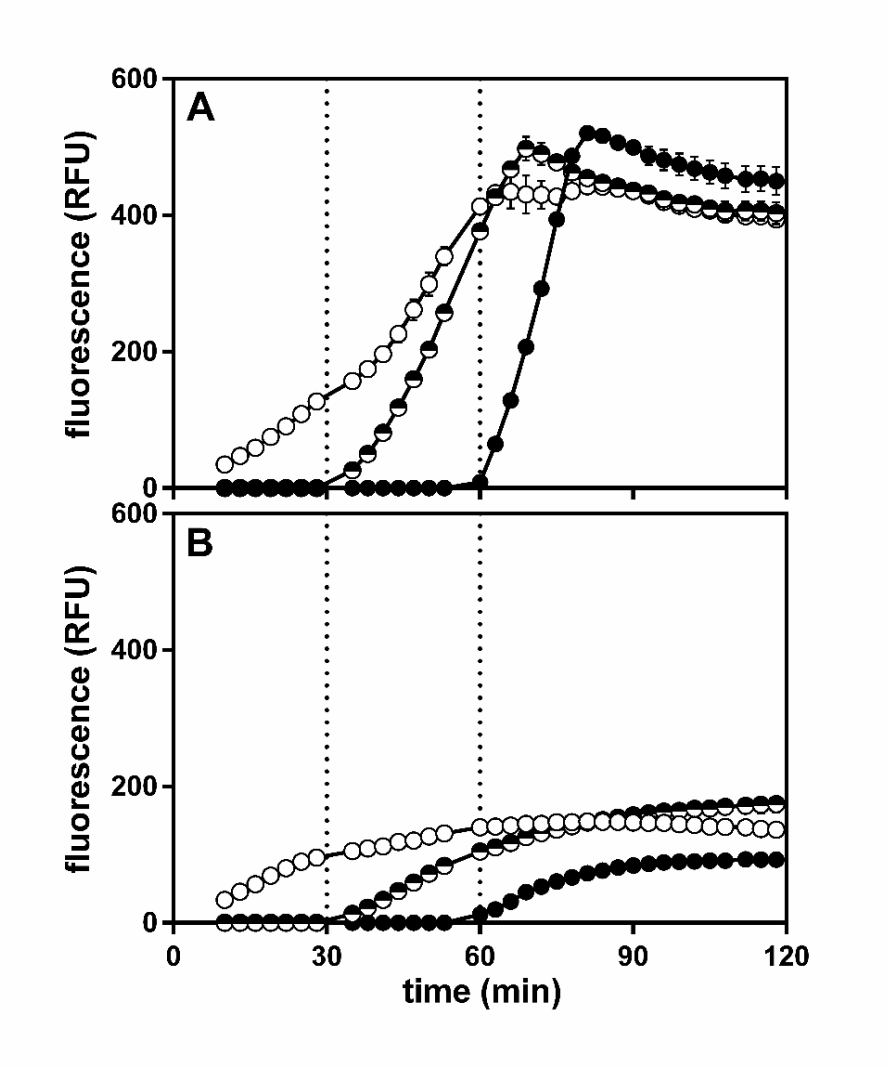


Fig 9. Effect of the time of resazurin addition on the fluorescence kinetics of control and phage-infected cultures of *E. coli*. Resazurin was added at 0, 30 and 60 min (indicated by a vertical dotted line). Evolution of fluorescence along time for: 10^8 cfu/mL *E. coli* culture without phages (A), and 10^8 cfu/mL *E. coli* culture exposed to $5x10^7$ pfu/mL of T4 phage (B). Error bars represent the standard error of each measurement.

Regarding the effect of cell concentration on the sensitivity of the assay, Fig. 10 shows the results of the three of the bacterial concentrations tested (10^8, $10^7$and 10^6 cells/mL) when exposed to concentrations of phage ranging from $5x10^1$ to $5x10^8$ pfu/mL. Results from the remaining phage/bacteria combinations can be found in Supplemental Fig. S2 and in Dataset S2. Fig. 10A, displays the evolution of fluorescence over time for a 10^8 cfu/mL *E. coli* culture when exposed to different T4 phage concentrations. As can be seen, samples treated with $5x10^6$ pfu/mL and lower did not differ from the control since resazurin was completely reduced before phage

proliferation had the chance to impact culture activity in a detectable way. In all cases fluorescence increased during the first 60 min until reaching maximum values that corresponds to complete reduction of resazurin. Addition of phage at $5x10^8$ pfu/mL resulted in an initial increase in fluorescence that stopped abruptly after 30 minutes, coinciding with the complete lysis of the culture. Lowering phage concentration down to $5x10^7$ pfu/mL allowed for a further increase in fluorescence as the culture took longer to lyse. However, maximum fluorescence remained considerably below the levels of the control.

Identical experiments carried out using cell concentrations 10 and 100 times lower (10^7 and 10^6 cells/mL) have been represented in Figures 10B and 10C. The results of these experiments indicate two very clear trends: First, decreasing cell concentration affects the length of the lag period before either growth or lysis can be detected using fluorescence. With 10^8 cells/mL (Fig. 10A) this lag phase is virtually inexistent while with 10^7 cfu /mL it extends to 90 min (Fig. 10B), and with 10^6 cells /mL it reaches 150 min (Fig. 10C). Second, the use of lower cell concentrations increases the sensitivity of the assay as it allows phage propagation before resazurin is completely reduced by the culture. While 10^8 cells/mL allowed the detection of phages at concentrations higher than $5x10^6$ pfu/mL (Fig. 10A), using 10^7 cells /mL lowered the detection limit to > 10^5 phages/mL. When cell concentration was lowered to 10^6 cells/mL sensitivity increased even further, allowing the detection of phage numbers as low as 10^1 pfu/mL. Again, this is possible because the long time required for 10^6 cells/mL to grow and completely reduce resazurin, allows also amplification of the phage when present at low initial concentrations. As a rule, lower cell numbers allow for higher sensitivity but at the expense of longer incubation times, similar to what has been previously reported (Rajnovic et al. 2019).

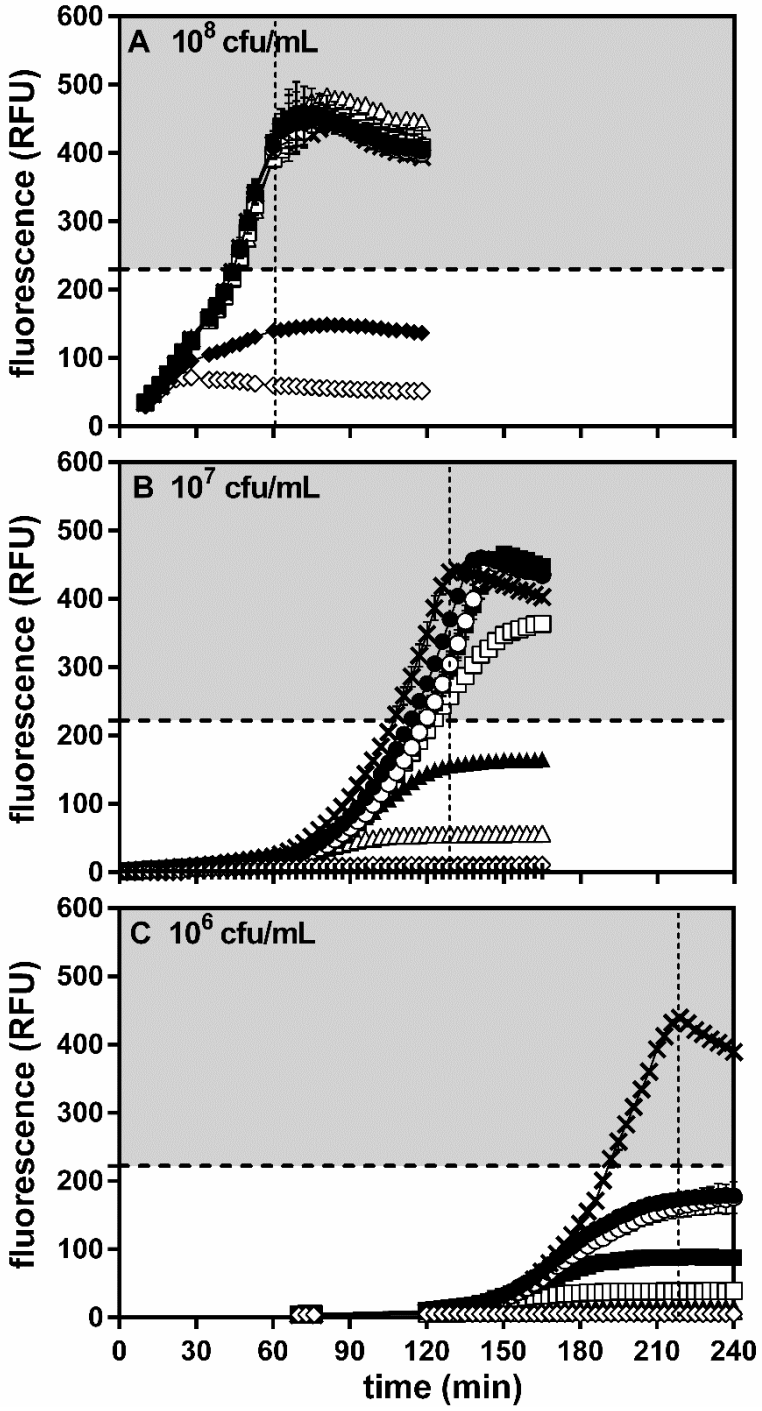


Fig 10. Fluorescence kinetics in cultures of *E. coli* exposed to different T4 phage concentrations. Several concentrations of bacteria: 10^8 (A), 10^7 (B), and 10^6 (C), were tested against different phage concentrations: $5x10^8$ (◇), $5x10^7$ (◆), $5x10^6$ (△), $5x10^5$ (▲), $5x10^4$ (□), $5x10^3$ (■), $5x10^2$ (○), $5x10^1$ (●) and 0 pfu/mL (×). Error bars represent the standard error of each measurement.

In order to systematically assess how the phage detection threshold and the length of the assay change as a function of cell concentration, we have extracted the Time of Detection and detection thresholds from Supplemental Fig. S2 and plotted them in Fig. 11 for each of the cell concentration tested. The results confirm the conclusions drawn from Fig. 10. The length of the assay, as indicated by the Time of Detection (ToD), decreases when increasing cell concentration. At the lowest cell concentration tested (10^5 cfu/mL), ToD is 310 min. This time decreases at a rate of ~80 min per every 10-fold increase in bacterial cell concentration, down to 30 min. The assay cannot get any shorter because T4 requires approximately 30 minutes from infection to lysis (Hadas et al. 1997). On the contrary, the phage detection thresholds decrease when decreasing cell concentration, down to $5x10^1$ pfu/mL which was the lowest concentration tested. This trend is interrupted at 10^6 cells mL mainly because we did not test concentrations lower than $5x10^1$.

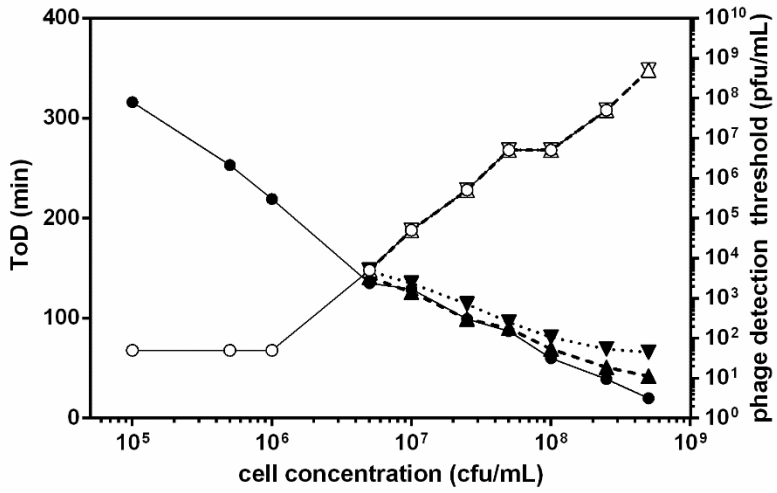


Fig 11. Representation of the Time of Detection (ToD) (○) and phage detection thresholds (●) as a function of the bacteria concentration used in the experiment. ToD is the time at which the controls without phages reach maximum fluorescence and it depends on the concentration of bacteria initially present in the culture. Phage detection threshold is the minimum concentration of phage that can be detected at ToD for a certain cell concentration. The results, indicated by a solid line, were obtained in experiments in which resazurin was added at time zero. The experiments were repeated for cell concentrations between $5x10^6$ and $5x10^8$ cfu/mL, adding resazurin at time 30 minutes (dashed line, ToD (△), phage detection thresholds (▲)) and also at time 60 minutes (dotted line, ToD (▽), phage detection thresholds (▼)).

The reason for this is that the microwell format adopted forced us to use a 20 µL volume for the phage samples. A concentration of 50 phage/mL in a reaction mix of 200 µL corresponds to a total of 10 phages in the 20 µL phage sample. Concentrations of phage 10 times lower would mean that 20 µL samples contain only 1 phage. At these concentrations the reliability of the assay breaks down, as the probability of having void samples, calculated according to equation 5 (see 3.2 Materials and Methods) increases considerably up to 0.369. This means that, under the conditions used, one third of the assays carried out containing 5 phages per mL would show absence of phages. This could be overcome, and the sensitivity of the assay increased, either by modifying the assay to allow larger sample volumes, or by increasing the number of replicates to account for the probabilistic limitations of the method.

The experiments were repeated for cell concentrations between $5x10^6$ and $5x10^8$ cfu /mL, adding resazurin at time 30 minutes and also at time 60 minutes (Fig. 11). Phage detection thresholds were not affected by the time at which resazurin was added, with one exception. At the highest concentration of bacteria ($5x10^8$ cfu/mL) addition of resazurin at time zero did not allow phage detection, as resazurin was completely reduced before the first cells started to lyse (latent period of T4 is ca 30 minutes (Hadas et al. 1997)). When resazurin was added after 30 and 60 minutes, lysis of the culture was extensive enough to allow detection of $5x10^8$ pfu/mL and higher. Regarding the Time of Detection (ToD), a slight increase was observed when resazurin was added at 30 and 60 minutes, which resulted in an overall increase in the length of the assay. Summarizing, decreasing concentration of bacteria in the reaction mix improves detection at low phage titers, but at the expense of lengthening the assay considerably.

This can be appreciated perhaps more clearly in Fig. 12 in which the length of the assay has been plotted as a function of the phage detection threshold. Detecting $5x10^1$ phages per mL requires approximately 3.5 hours when using 10^6 cells/mL. Aiming at a lower detection limits ($>10^7$ pfu/mL) allows decreasing the length of the assay considerably, down to 40 minutes. Both, phage detection threshold and length of the assay are subject to methodological constrains. The lowest phage detection threshold, shown as a vertical dashed line results from the sample volume

limitation discussed above, and could be overcome by designing an assay format able to process larger volume samples. The shortest assay length, indicated by a horizontal dashed line, is a consequence of the minimum time required by the phage to infect the cells and lyse them. In the case of T4 this corresponds to approximately 30 minutes (Hadas et al. 1997).

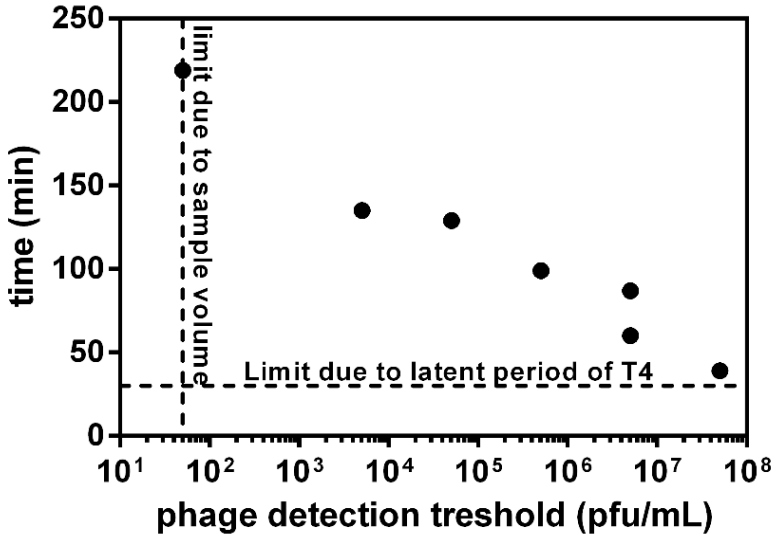


Fig 12. Relationship between detection threshold and duration of the assay. As a rule, increasing the desired detection threshold reduces the length of the assay**.** Dashed lines indicate the main constrains of the assay. The vertical dashed line indicates the limit of detection imposed by the sample size used. The horizontal dashed line corresponds to the shortest possible assay length, conditioned by the time required for the phages to carry out their lytic cycle.

Finally, in order to check the performance of our fluorescence assay in media with different turbidities we used the combinations of cell-phage concentrations described above, and tested them over samples supplemented with turbidity standards (synthetic polymer beads) at turbidity levels of 1000 NTU and 2000 NTU. In order to check the validity of the measurements in a more complex matrix, we carried also measurements in samples supplemented with milk.

The results of these measurements are shown in Fig. 13. The control without turbidity (0 NTU) has already been represented in Fig. 10B and therefore is not included in Fig. 13. Fluorescence measurements carried out with standards of different turbidity (1000 and 2000 NTU) (Fig. 13A and 13B) are very similar to the results found in the 0 NTU samples. As before, samples with

concentrations of phage $\geq 5x10^5$ phages/mL display little or no fluorescence, well below the threshold limit. Samples with less than $5x10^5$ pfu/mL have fluorescent kinetics similar to the control, always above the threshold limit of detection. In the case of milk (Fig. 13C), a slight difference in behavior can be noticed as fluorescence increases somewhat faster and suffers a slight decrease at lower phage concentrations.

The overall impression is that, in the assay carried out in the presence of milk, microbial activity was higher, maybe as a result of the availability of additional nutrients not found in the culture medium used. This higher activity results in steeper slopes, a slightly shorter Time of Detection, and a decrease in fluorescence when the fluorescent product of resazurin reduction, resorufin, was further reduced to non-fluorescent dihydroresorufin. Regarding the ability to discriminate different phage concentrations, the assay provided consistent results both, in clear samples, and in samples of different turbidity levels.

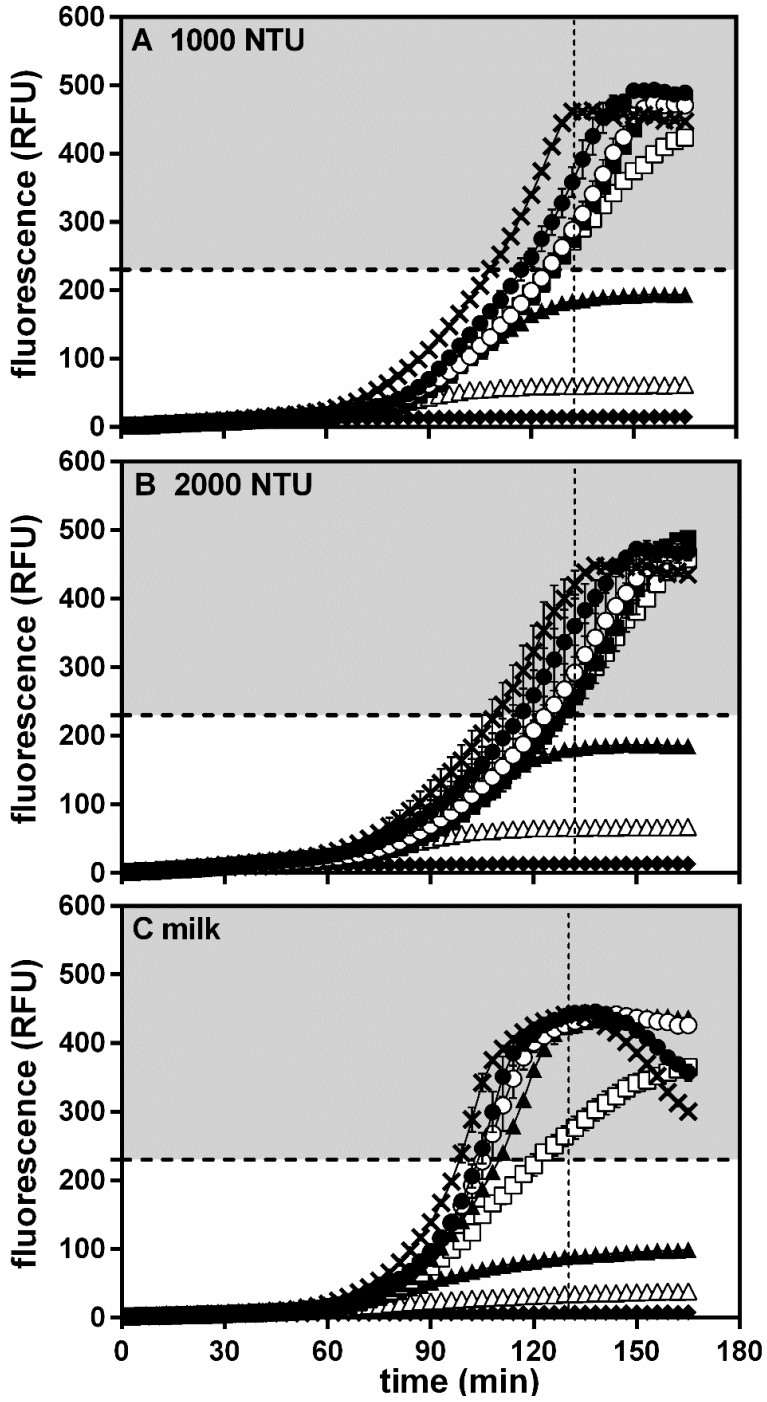


Fig 13. Kinetics of fluorescence in cultures of *E. coli* exposed to different T4 phage concentrations in samples of different turbidity grades and in milk. Different phage concentrations ($5x10^8$ (◇), $5x10^7$ (◆), $5x10^6$ (△), $5x10^5$ (▲), $5x10^4$ (□), $5x10^3$ (■), $5x10^2$ (○), $5x10^1$ (●) and 0 pfu/mL (×)) were tested against *E. coli* cultures containing 10^7 cfu/mL, under several turbidity grades and matrix (1000 NTU (A), 2000 NTU (B) and milk (C)). Error bars represent the standard error of each measurement. Data corresponding to the same set of bacteria and phage concentrations measured at 0 NTU are represented in Fig. 10B.

In terms of detection threshold and assay time, the method proposed performs similar or better than several of the methods currently available for phage detection. A comparison with these methods has been summarized in Table 10. The sensitivity of these methods spans across several orders of magnitude. In the lower sensitive range, some high-end methods like TEM (Zhang et al. 2013), MALDI-TOF (Stverakova et al. 2018) and flow cytometry (Oliveira et al. 2017) do not provide sensitivity greater than 10^5 pfu/mL, but results are obtained in a short period of time, under 2 h. These methods require expensive equipment and highly qualified personnel to operate them and are generally subjected to sample pre-treatment when processing turbid samples. For instance in the case of flow cytometry, measuring turbid samples such as milk requires some type of pre-treatment, as fat and eukaryotic cells must be removed to prevent clogging (Michelsen et al. 2007). Use of high-end methods with better sensitivity (10^2 pfu/mL) such as SPR (Garcia-Aljaro et al. 2008) and Raman spectroscopy (Tayyarcan et al. 2018), requires dilution or complete removal of the matrix when dealing with turbid samples such as blood, serum and milk, in order to eliminate its effect on the measurements (Tayyarcan et al. 2018, Shankaran et al. 2007). In a different category, DNA based methods are probably the most common solution for fast phage monitoring as they are sensitive enough for the detection and quantification of low phage concentrations (10^3-10^2 phages/mL) in a short period of time of under 2.5 h. However, their use in turbid samples requires a pre-treatment to remove the turbid matrix. In the case of qPCR this matrix must be discarded because it contributes to inhibit the PCR reaction, adding an extra 30 minutes to the length of the assay (Ly-Chatain et al. 2011) . Multiplex PCR has been successfully used to analyse the presence of phages in turbid samples without sample pre-treatment but at the expense of reducing considerably the sensitivity and bringing the detection limit up to 10^5 pfu/mL (Martin et al. 2008).

Table 10. Performance of different phage detection methods currently available.

Methods	sample type	LOD (pfu/mL)	ToD (h)	references
Resazurin reduction	polymer beads and milk	$5x10^1$	3.5	this work
Flow Cytometry	synthetic	10^5	1	Oliveira et al. 2017
Surface Plasmon Resonance (SPR)	wastewater	10^2	2	Garcia-Aljaro et al. 2008
MALDI-TOF	synthetic	10^7	2	Stverakova et al. 2018
Raman spectroscopy	milk	10^2	1	Tayyarcan et al. 2018
qPCR	milk and whey	10^2	2	Ly-Chatain et al. 2011
qPCR	milk	10^5	0.5	Martin et al. 2008
Gel Loop-mediated isothermal amplification (gLAMP)	wastewater	10^3	1	Huang et al. 2018
Paper-based ELISA	synthetic	10^3	2	Khan et al. 2015
Release of intracellular component	water	10^1	3	Muniesa et al. 2018, Ijzerman et al. 1993
ATP release	synthetic	10^1	3	Guzman Luna et al. 2009
OD kinetics	synthetic	$5x10^1$	3.5	Rajnovic et al. 2019
Long-period grating sensor	synthetic	$5x10^3$	0.5	Janczuk-Richter et al. 2017
Impedance measurements	milk	10^2	6	Garcia-Aljaro et al. 2009
Capacitive measurements	river water	10^1	1	Erturk & Lood 2018
Voltammetry	reservoir water	10^1	0.5	Reta et al. 2016
QuantiPhage assay	wastewater	10^1	3	Rames & Macdonald 2019
Water Oil Water emulsion microdroplets	synthetic	10^2	1	Wang & Nitin 2014
TEM	any treated	10^5	1	Zhang et al. 2013
Double Agar Layer	any diluted	10^1	18	Kropinski et al. 2009, Adams 1959

Finally, higher sensitive methods like QuantiPhage (Rames and Macdonald 2019), release of intracellular components (Muniesa et al. 2018, Ijzerman et al. 1993) and electrochemical (capacitive and voltammetry) measurements (Erturk and Lood 2018, Reta et al. 2016) can detect 10^1 pfu/mL within 3 h. The interference of the turbidity in these methods haven't been evaluated yet, as they are mainly used to detect coliphages in water. Other methods mentioned have sensitivities in the 10^2-10^3 pfu/mL range with times of detection between 1-6 h.

From the point of view of sensitivity, the approach that we describe in this chapter allows the detection of $5x10^1$ phages per mL in 3.5 h, placing it the top sensitivity tier when compared to

other high-end methods. The time required to obtain measurements is perhaps somewhat longer but, as a plus, it uses relatively simple technology. In our case we have used a microplate fluorimeter, but being based on a threshold type criterium, the method can be easily translated to semiquantitative format in which fluorescence could be detected using a hand held UV lamp.

As discussed above, and in a previous chapter the sensitivity of our method is limited to $5x10^1$ pfu/mL as a consequence of the small sample volume used in the microplate assay. Working with small volumes increases the probability of having void samples when dealing with low phage concentrations. This can be improved by increasing the number of replicates, but much more efficiently by modifying the assay in a way that accepts larger sample sizes. According to equation 5, which represents the mass function of the Poisson probability distribution for the particular case of having a sample with zero phages, the concentration at which the reliability of the assay breaks down due to the appearance of void samples, decreases tenfold per every tenfold increase in sample volume. Thus, if using 20 µL samples allows a detection limit of 500 phages per mL of sample, increasing this volume to 200 µL would allow detection of 50 phages per mL, and working with 2 mL samples would allow detection of 5 phages per mL.

In summary, the method described allows fast and sensitive detection of phages in liquid samples through the measurement of phage-induced changes of activity in growing cultures of the host organism, using resazurin as a reporter. The method, which is well suited for miniaturization, automatization and high throughput analysis, is not affected by the turbidity of the sample, uses non-sophisticated technology and provides a simple and inexpensive solution when compared to other high-end methods.

3.4 Conclusion

Fluorescence monitoring of resazurin reduction in growing cultures of *E. coli* exposed to phage-containing samples, allows for fast and efficient detection of bacterial viruses. The concentration of bacteria initially present in the culture determines both the phage detection level as well as the length of the assay. As a rule, low concentrations of cells allows to detect low concentrations of

phages, but with long detection times (e.g. an initial concentration of 10^6 cells/mL allows detection of 10 phages in 3.5 hours). Increasing cell concentration reduces the length of the assay, but at the expense of decreasing the detection threshold (e.g. $2.5x10^8$ cells/mL shortens the assay to 40 minutes but increases the detection threshold up to 10^7 phages). The assay doesn't require any pre-treatment of the sample, and is quite robust in the sense that the outcome is not affected to a significant extent by the time at which resazurin is added (0, 30 or 60 minutes), neither by the amount of turbidity present in the sample (tested at 1000 NTUs, 2000 NTUs and using milk).

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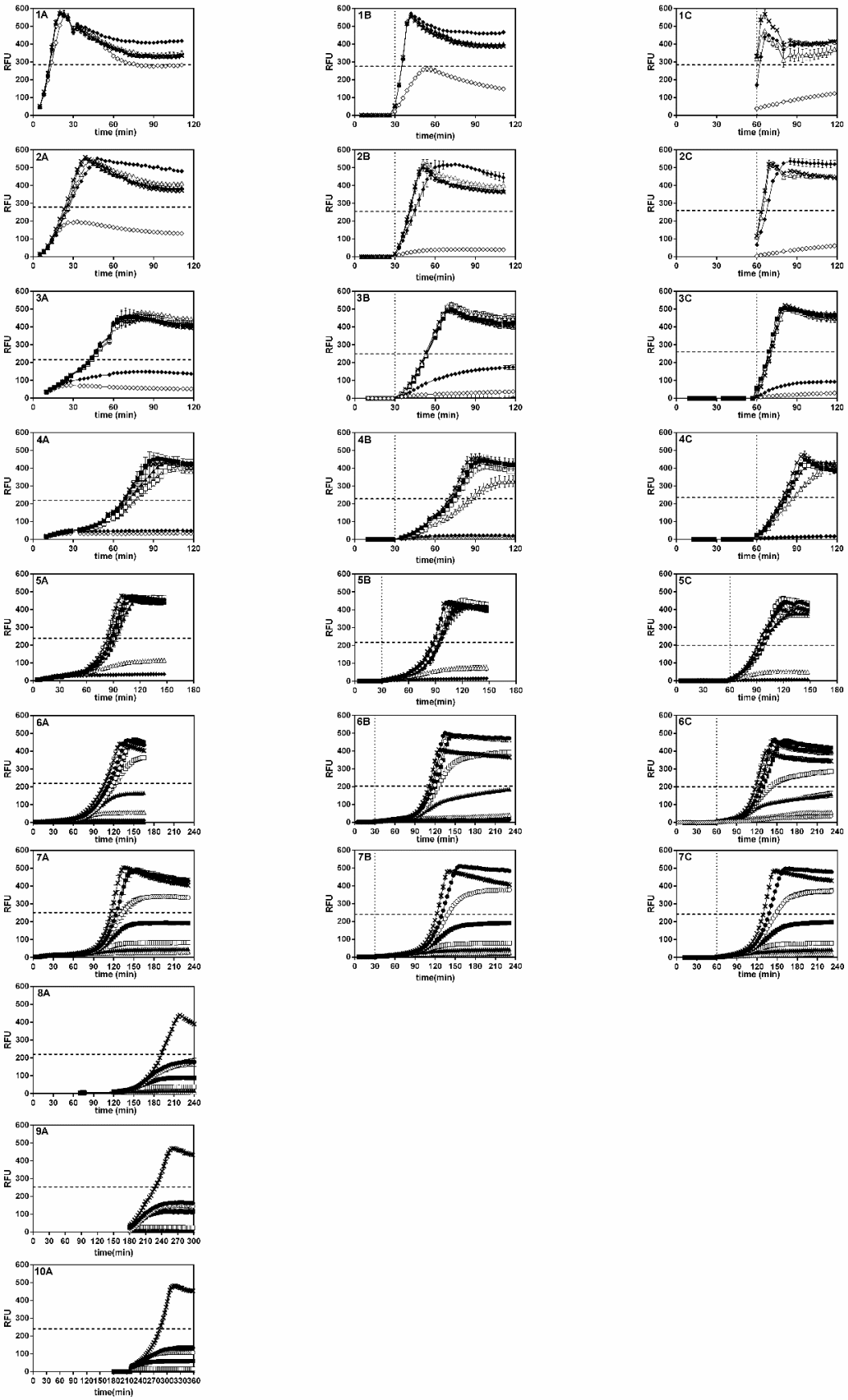
3.5 Supporting information



Supplemental Fig. S2. Kinetics of fluorescence in cultures of *E.coli* exposed to different T4 phage concentrations. 10 bacteria concentrations: $5x10^8$ (**1**), $2.5x10^8$ (**2**), 10^8 (**3**), $5x10^7$ (**4**), $2.5x10^7$ (**5**), 10^7 (**6**), $5x10^6$ (**7**), 10^6 (**8**), $5x10^5$ (**9**) and 10^5 (**10**) were tested over different phage concentrations: $5x10^8$ (◇), $5x10^7$ (◆), $5x10^6$ (△), $5x10^5$ (▲), $5x10^4$ (□), $5x10^3$ (■), $5x10^2$ (○), $5x10^1$ (●) and 0 pfu/mL (×). The experiments were carried out adding resazurin at time zero (**A**), 30 minutes (**B**) and 60 minutes (**C**). Error bars represent the standard error of each measurement.

S2 Dataset contains the fluorescence vs time data corresponding to 186 different phage/host combinations. Data have been used to create the graphs in Supplemental Fig. S2. A subset of the data has been used for Figures 9, 10, 11 and 12. The complete dataset can be found in 5.2.1 Annex.

4. General Discussion

The development of phage detection and quantification methods is highly influenced by several factors: 1) type of phage; 2) level of sensitivity required; 3) availability of cultivable hosts; 4) possible interference by the sample matrix; 5) availability of sophisticated equipment and/or highly skilled personnel and, conditioning most of the above, 6) type of application (phage therapy, bio-control studies, food fermentation industry or environmental monitoring).

The work developed in this thesis explores the possibility of carrying out sensitive detection and quantification of specific phages, by **analyzing the dynamic behavior of the phage-host system**. The thesis neither describes the development of methods for specific applications, nor attempts to provide solutions to particular problems, but rather constitutes a basic study using *E.coli* and T4 as model organisms to provide the **conceptual framework** for phage detection, the **proof of concept**, and a **very extensive set of data**. All of these elements can be used as the basis for the development of specific methods tailored to any of the areas mentioned above.

In the first phase we have monitored optical density changes during phage-induced culture lysis in clear media in order to evaluate phage-host kinetics over different combinations of bacteria/phage concentrations. In a second phase the fluorescent properties of the redox dye resazurin have been employed to overcome the problems observed when attempting to perform optical density kinetic measurements in media of high turbidity. Thanks to the fluorescent properties of resazurin, the phage-host kinetics of different combinations of bacteria/phage concentrations have been evaluated in synthetic turbid media as well as in a high complexity matrix such as milk.

Optical density (OD) measures the amount of attenuation or scattering when light of a certain wavelength passes through a medium. Bacteria inside such medium possess light scattering properties, and in sufficient numbers they contribute to the “cloudy” or “milky” appearance usually referred to as turbidity (Sutton 2006). OD-based assessments of a bacterial concentration is usually performed at a wavelength of 600nm (OD_{600}) and requires a previous calibration to relate OD_{600} values to cell concentration. This calibration is not universal and must be carried out for each organism as they can differ in size, mass and may have pigments (Sutton 2006). Also, the calibration can be affected by the spectrophotometer used, the size and conditions of the detectors,

the width of the optical slit, the condition of the filter and total output of the light source as they may differ depending on the spectrophotometer model. Ideally, the most sensitive spectrophotometer would have a narrow slit and a small detector that only detects light scattered in forward direction.

OD-based monitoring of growth in actively bacterial cultures indicates the existence or four different stages: 1) lag phase (no visible growth), 2) log phase (exponential growth), 3) stationary phase (no growth) or 4) death phase (Mandelstam et al. 1982), as shown in Figure 14.

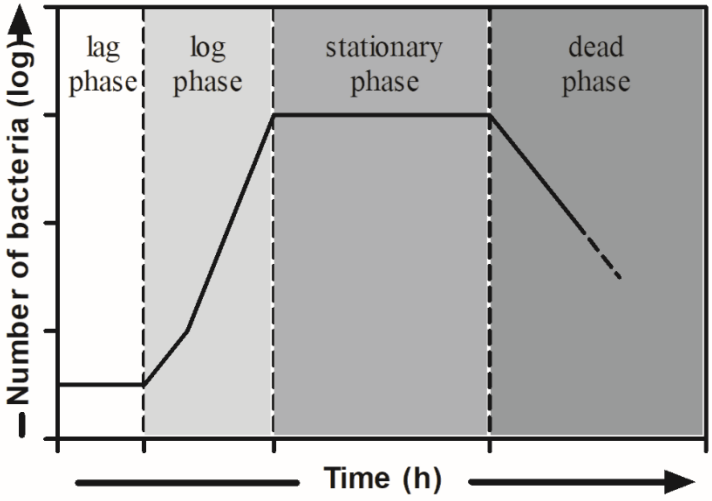


Figure 14. Illustration of bacterial growth and growth stages

In the absence of T4, *E.coli* cultures grow unimpaired and the kinetics follow the expected pattern before reaching stationary phase. This is also the case when the initial phage concentration in the sample is very low, and cultures reach the stationary phase before phages have any chance to increase their number to significant levels as shown in Fig 15a. When bacterial cultures enter stationary phase cells are no longer actively growing thus becoming “safe” for low phage numbers, because phage replication and production of viral particles requires an active metabolism. However, this “safe” status can be compromised if the concentration of phages introduced into the system is higher than the concentration of bacteria.

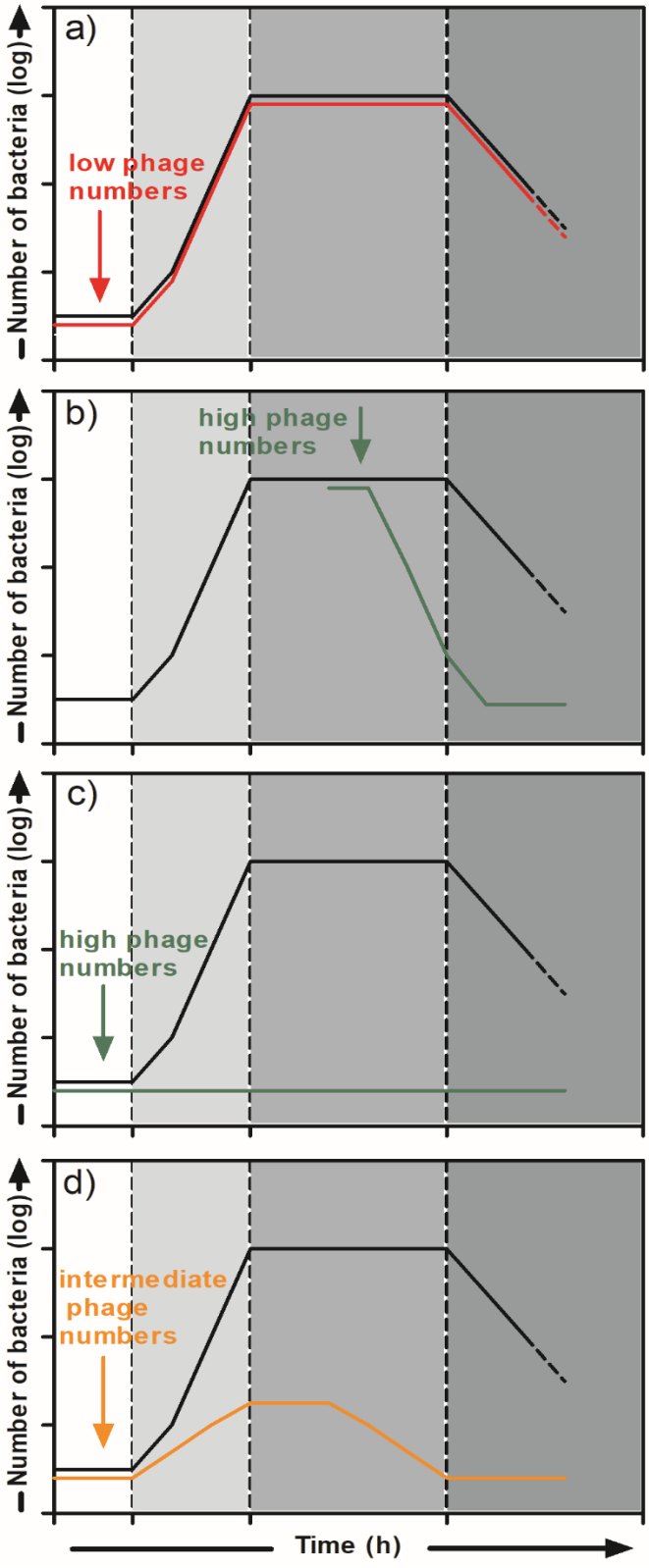


Figure 15. Illustration of 4 possible phage-host outcomes. a) growth curve of phage-infected culture when low phage concentration is added at the beginning of the experiment (red line); b) effect of added high phage concentration (at least 1 order of magnitude higher than bacteria) when in the stationary phase (green line); c) growth curve of phage infected culture when higher phage concentrations (at least 1 order of magnitude

higher than bacteria) are added at the start of the experiment; d) “abnormal” growth curve of phage-host infected culture when intermediate phage concentration is added at the beginning (orange line). In all cases black line present normal growth curve.

This event will trigger multiple phage infections of a single bacteria cell which will eventually lead to instant lysis and a rapid decrease in turbidity even in inactive bacteria, in a phenomenon called "lysis from without" (Abedon 2011), Figure 15b. In cases in which phages outnumber bacteria in the lag phase, no detectable growth is visible (Fig 15c) as cells are completely lysed before growth becomes detectable. In conditions in which lysis occurs during the exponential phase an “abnormal”, accelerated event of growth stages and premature death can be seen as in Figure 15d, which indicates that the initial concentration of phages in the sample was high enough to lyse the bacteria culture before reaching the stationary phase. The size and shape of these “abnormal” (accelerated and premature death curves) depends on phage characteristics such as latent period and burst size. This values of these variables for some representative phages are presented in Table 11.

Table 11. Table of bacteriophage species with related latent period and burst size

bacteriophage	latent period	burst size	references
λ	42 min	115	de Paepe & Taddei 2006
MS2	40 min	5000-10000	de Paepe & Taddei 2006, Loeb & Zinder 1961
M13	30 min	continuous release	Campbell (unknown)
T2	23 min	135	de Paepe & Taddei 2006
T3	17 min	200	de Paepe & Taddei 2006
T4	23 min	150	de Paepe & Taddei 2006
T5	44 min	290	de Paepe & Taddei 2006
T7	13 min	260	de Paepe & Taddei 2006
ΦX174	15 min	180	de Paepe & Taddei 2006
PRD1	48 min	500	de Paepe & Taddei 2006, Bamford et al. 1981
MA-LMM01	6-12 h	50-120	Yoshida et al. 2006
A2	140 min	180-200	Yoshida et al. 2006
ΦY8	30 min	100	Villion & Moineau 2009

Bacteriophage T4 has a burst size of 150 phages and a latent period of approximately 25 min. This means that every 25 min phages increase their numbers by a factor of 150 with clear impact on culture kinetics, as can be clearly seen in Figure 16 in which the event of lysis has been divided in three steps: a) first burst or first detectable lysis; b) latent period and c) final burst.

Careful analysis of the optical density kinetics of 90 different combinations of phage-host concentrations has provided a set of calibrations that allow to estimate the amount of phage present in a sample as a function of the percent inhibition expressed on a curve area basis. These calibrations provide different dynamic ranges depending on the concentration of host bacteria used. The dataset has been published and made available to the community as it constitutes probably the first detailed study on phage-host concentration dependence that hasn't been done from the discovery of bacteriophages in the 1920's.

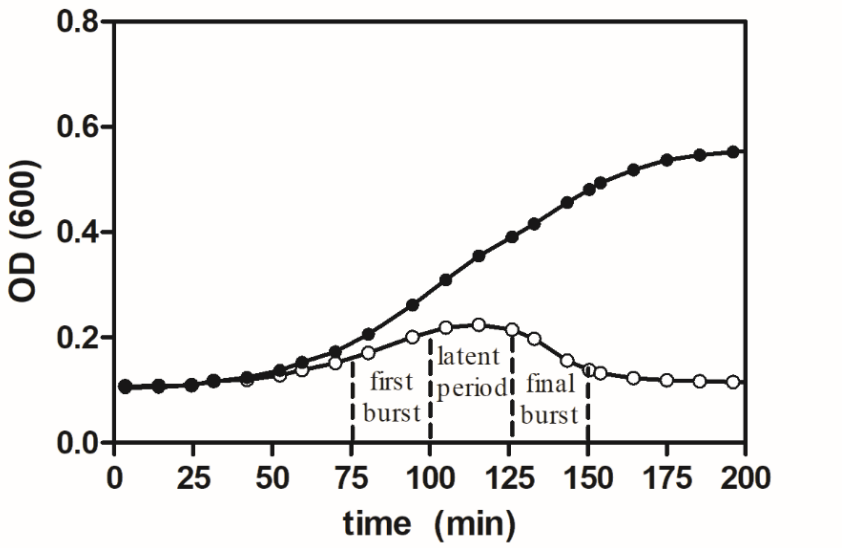


Figure 16. Stages of lysis during a phage-host infection. Growth curve of an actively growing *E.coli* culture of 10^7 without bacteriophages (●). Lysis stages that are only visible during an "abnormal" growth event, and lysis stages of 25 min matches with latent period of bacteriophage T4 (○). The addition of phages at concentration of $5x10^5$ pfu/mL happened at time 0.

The effectivity to detect and quantify phages by this approach can be impaired in high turbid samples such as blood, body fluids, turbid water and fermentation samples in which OD measurements are affected by the extremely high degree of light scattering inherent to these matrices. Under these conditions it is virtually impossible to monitor bacterial growth or lysis through optical density without any pre-treatment steps. To overcome the inconvenients related to high turbidity samples we have developed a second approach that fulfills the requirements of being simple, fast and able to operate in complex turbid media while maintaining the same efficiency as

the OD based method. This approach was developed using resazurin, a non-toxic cell-permeable, redox-sensitive dye that is often used to assess the respiratory activity of metabolically active cells in a number of cell-based assays. Resazurin is an example of a much broader category of the so-called viability or activity dyes, some of which are presented in Table 12.

Table 12. Table of commercially available cell viability dye

Cell viability dye	oxidized form color	reduced form color	references
Resazurin	blue	pink-fluorescent	Rajnovic & Mas 2020
Methylene blue	blue	colorless	Bapat et al. 2006
Neutral red	red	colorless	McKinlay & Zeikus 2004
Viologen	colorless	blue	Aulenta et al. 2007
MTT	yellow	purple	Patel et al. 2013
TTC	colorless	red	Tengerdy et al. 1967
CTC	colorless	orange-fluorescent	Schaule et al. 1993
INT	colorless	red	Dufour & Colon 1992
WST-1	colorless	red	Orman & Brynildsen 2013
XTT	colorless	orange	Roslev & King 1993
Propidium iodide	colorless	red-fluorescent	Wang & Nitin 2013

In its oxidized form resazurin is weakly fluorescent and purple to blue in color. In the presence of respiratory activity this compound is irreversibly reduced to pink resorufin which is highly fluorescent. The detection of resorufin fluorescence is achieved at excitation wavelength between 530-570 nm with an emission maximum at 580-590 nm (Chen et al. 2015, Rajnovic and Mas 2020). The choice of a fluorescent substrate to carry measurements in turbid samples is due to the fact that fluorescence is less prone to be attenuated than optical density when working in turbid media. Actually, fluorescence-based measurements can even be carried out with good sensitivity using solid substrates such as in paper-based assays. In this case the assay detected the increase in metabolically reduced resorufin as the culture grew and cell biomass increased. In non-infected cultures fluorescence kinetics mimic the behavior of OD based growth curves as shown in Figure 17.

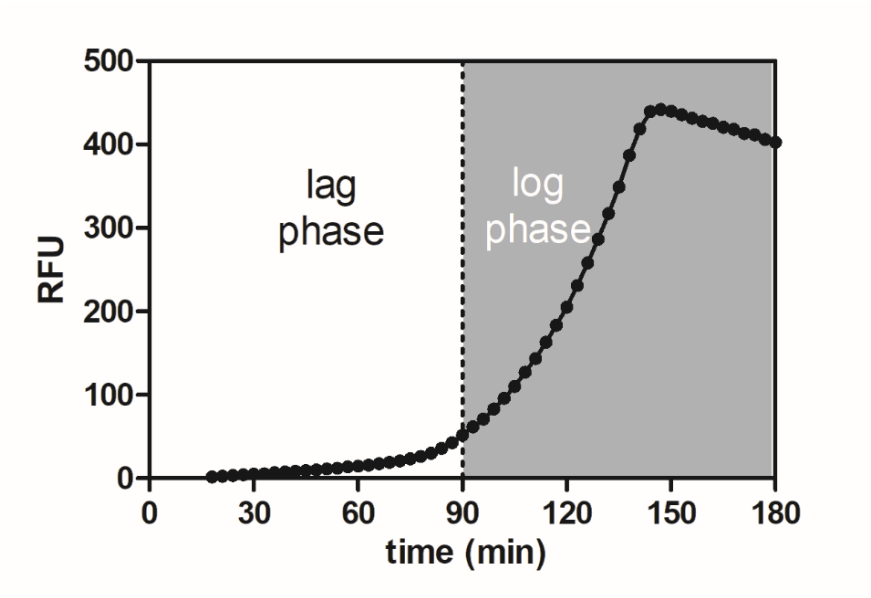


Figure 17. Fluorescence kinetics of an actively growing bacteria culture which converts the cell viability dye resazurin to fluorescent resorufin.

After the initial lag phase fluorescence increases during the exponential growth phase until reaching a plateau indicating that the culture converted all available resazurin. The small drop in fluorescence afterwards indicates that the culture is still metabolically active and able to reduce resorufin further to nonfluorescent dihydroresorufin. Anyhow, the experimental design of the assay is strictly focused on the relation of nonconverted resazurin and resorufin at specific time ranges, thus eliminating interference of dihydroresorufin in the late log and stationary phases. Figure 18 shows the model kinetics of bacterial cultures infected with different amounts of phages in the early log phase when growing in optically clear culture medium.

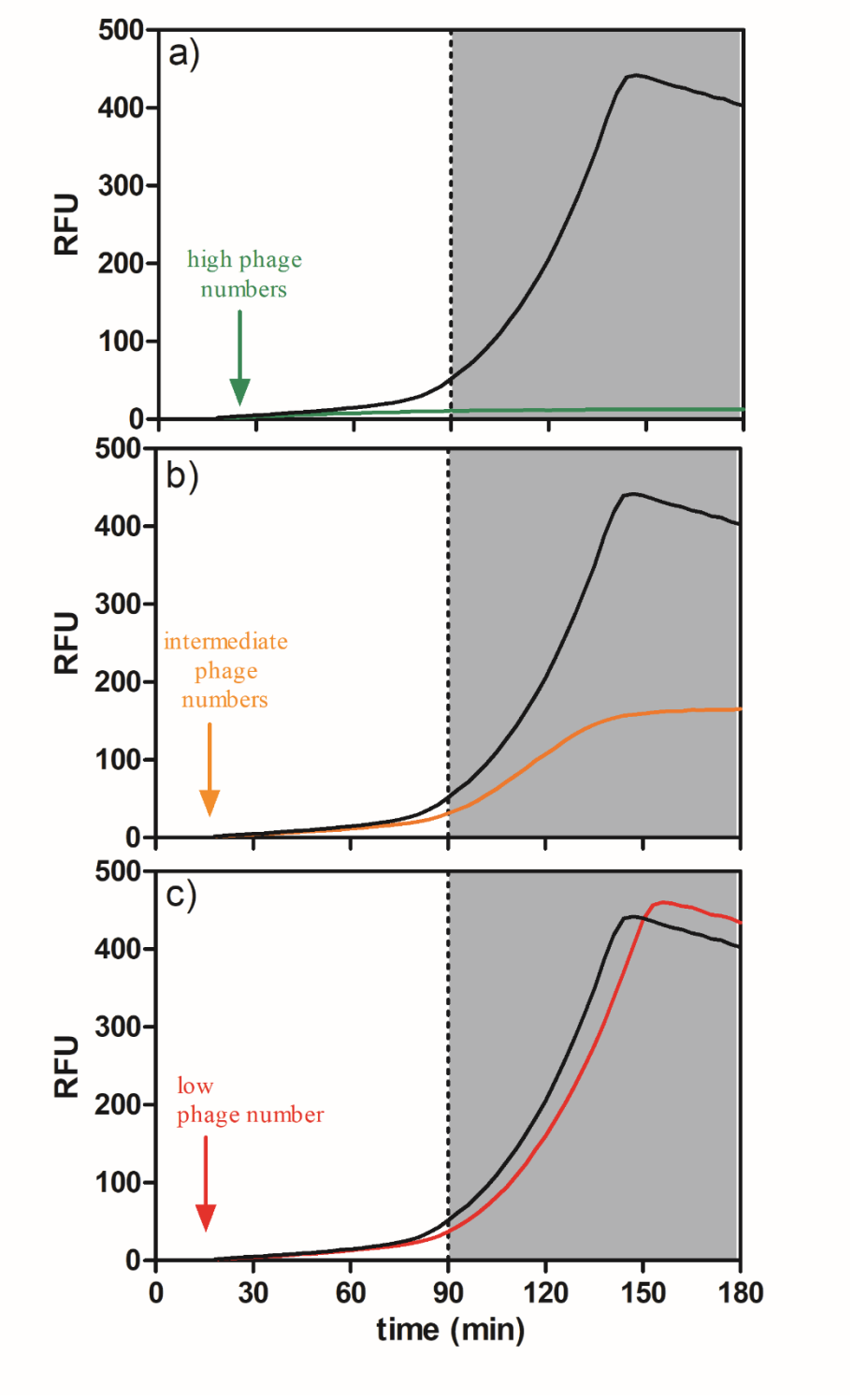


Figure 18. Illustration of 3 possible phage-host outcomes. a) fluorescent growth curve of a phage-infected culture when (a) a large amount of phage (at least 1 order of magnitude larger than the amount of bacteria) is added at the beginning of the experiment (green line), (b) an intermediate amount of phage is added at the start and lysis happens during the log phase (orange line) and (c) a small amount of phage is added at the beginning of the experiment insufficient to induce visible lysis before the culture reduces all the substrate to the fluorescent form (red line). In all cases black line represent normal fluorescence kinetics of an actively growing bacterial culture without bacteriophages.

In Figure 18a, a flat or weak fluorescent signal is present throughout the entire assay indicating that phages outnumbered the initial population of bacteria in the sample. In Figure 18b lysis occurs during the log phase and the formation of resorufin stabilizes due to absence of an actively growing culture. Finally, when phage concentrations are much lower than bacterial concentrations (Figure 18c) the infected culture showed fluorescent curves similar to the control. The resazurin phage-host kinetics was further evaluated in synthetic turbid samples composed of latex beads and in milk. In all cases, phage host kinetics were similar, almost identical as those obtained with clear media which confirms the robustness of the assay.

When comparing both, optical density and fluorescence-based approaches, some common observations can be extrapolated. Both approaches suggest that using different initial concentrations of bacteria allows to define different dynamic ranges of phage detection with specific detection time-frames. The overview of detection limits and sensitivity ranges of both approaches are summarized in Table 13.

Table 13. Comparison of OD_{600} and fluorescent approach

	OD_{600}	Fluorescence
type of assay	quantitative	semi-quantitative
functional in turbid media	no	yes
fastest assay (min)	120	40
sensitivity range (pfu/mL)	$\geq 5x10^8$- $5x10^5$	$> 5x10^7$
most sensitive assay (min)	210	210
sensitivity range (pfu/mL)	$\geq 5x10^5$- $5x10^1$	$> 5x10^1$
largest sensitivity range	$> 5x10^6$- $5x10^2$	threshold values only

Both methods have their pros and cons as one is more quantitative but slower and the other semiquantitative but more robust. The OD-based method has better quantifying capacity but it is slower with a shortest time to detection of 120 minutes when using high bacterial concentrations (equal or higher than 10^8 cfu/mL) to detect phage contractions higher than $5x10^5$ pfu/mL. In the fluorometric approach the fastest detection can be achieved in just 40 min, using a high bacteria concentration as $2.5x10^8$ cfu/mL and a detection threshold up 10^7 pfu/mL or higher. At the highest sensitivities, both methods exhibit the same LOD and detection times, being able to detect $5x10^1$ pfu/mL in just 3.5 h by using low bacteria concentrations equal or lower than $5x10^6$ cfu/mL. The

LOD is highly affected by the small sample volumes (20 µL of sample in 200 µL of reaction mix. This corresponds to 10 phages in the well. Attempting to detect less than 10 phages per well is strongly hampered by the probabilistic effect of having false void samples. For instance, with 500 phages per mL the probability of drawing void samples when taking 20 µL is 1 in 22000. If the concentration is decreased tenfold to 50 phages the probability of void samples increases to 1 of each 3 decreasing the reliability of the assay to unacceptable levels. Decreasing the LOD down to 5 pfu/mL, would require either preconcentration steps or the ability to handle 2 mL samples, which is a normal assay volume for cell-based methods but rather cumbersome when attempting to develop high throughput low volume methods.

Concerning the categorization system that we used to categorize different phage detection and quantification methods in the introduction part of this thesis, our two approaches best fit under the section of “cell-based methods”. This is due to the fact that in both cases, phage detection is indirect, in other words, what is detected is the level of cell lysis of an active growing bacterial culture that is compromised by the presence of phages in the sample. The initial number of phages presented in the sample correlates with the degree of cell lysis that happens due to the replication and accumulation of phages during the assay. Regardless of whether detection is based on differences in optical density or in fluorescence, between phage-infected and non-infected cultures, neither of the approaches would work without a growing and metabolically active bacterial culture susceptible to act as the host and allow phages to replicate and accumulate in the sample. Therefore, we consider our proofs of concept first as “cell-based methods” rather than “spectroscopy-based methods”. When comparing our assays with the group of methods presented in the Introduction as “cell based methods” and summarized in Table 14, we can see that the LOD in our case is somewhat higher.

Table 14. Performance of different cell-based phage detection and quantification methods currently available.

Cell-based methods	phage species	sample type	sample pretreatment and analysis	sample pretreatment time	LOD (pfu/mL)	detection time	references
OD_{600} assay	coliphage T4	synthetic	no	no	$5x10^1$	3.5 h	Rajnovic et al. 2019
Resazurin assay	coliphage T4	synthetic, turbid synthetic and milk	no	no	$5x10^1$	3.5 h	Rajnovic & Mas 2020
Blue Phage (Beta Glucuronidase)	coliphages SOM3, SOM23 and ΦX174	synthetic, wastewater, river water, sludge and mussels	filtering of real samples	fast	$< 10^1$	2.5 h	Muniesa et al. 2018
Beta Galactosidase	coliphage Beta2	synthetic	IPTG induction	< 1.5 h	$< 10^1$	< 3.5 h	Ijzerman et al. 1993, Stanek & Falkinham 2001
ATP bioluminescence	coliphage ΦX17	synthetic	no	no	$< 10^1$	3 h	Guzman Luna et al. 2009
QuantiPhage (Beta Galactosidase)	coliphage ΦX174, MS2	surface water	no	no	$< 10^1$	3 h	Rames & Macdonald 2019
Latex agglutination culture enrichment method	F^+-specific RNA genogroups I,II,III,IV	synthetic	no	no	$< 10^1$	3 h	Love & Sobsey 2007

In all methods represented in Table 14 LOD is 10^1 pfu/mL and ´they can detect even 10^0 pfu/mL and our is $5x10^1$pfu/mL in both assays. This difference is due to sample volumes processed in each case, with most methods processing large sample volumes up to 100 mL while the total volume of our assays was 220 µL. However, as we indicated before, increasing the volume of the assay up to 2mL would increase the sensitivity while decreasing the LOD to $5x10^0$ pfu/mL as in the rest of methods. On the other hand many cell-based methods are highly dependent on time consuming pretreatment to the point that some of the methods are not functional without them. This is particularly true in cases in which enzymes, i.e. β-galactosidase or β-glucuronidase, have to be induced in active cultures in order to perform the assay properly, or when filtering prior to analyses is required to exclude turbidity from the sample. In our case we have proved that no sample pre-treatment steps are required and that the assays have a robust performance in samples of different turbidity grades. The absence of sample pretreatments improves the simplicity of the assay and decreases the time required to obtain reliable results making the assays proposed in this thesis an attractive alternative to currently existing methods.

The analytical procedure develop in this work compares also favorably to other groups of detection methods mentioned in the introduction of this thesis. The information for comparison is gathered from the tables of methods tables presented in Introduction of the thesis and is summarized in Table 15.

Table 15. Comparison and summary of groups of phage detection and quantification methods.

Group of methods	sample type (most common)	sample pre-treatment (most common)	sample pre-treatment time (most common)	LOD (most common)	Time of Detection (most common)
Cell-based	water samples	no	no	$\leq 10^1$ pfu/mL	< 210 min
DNA-based	milk and water samples	concentration and nucleic acid extraction	< 120 min	10^2-10^3 pfu/mL	< 180 min
Immunoassays	synthetic	no	no	10^4-10^7 pfu/mL	60-300 min
Electrochemical-based	synthetic and water samples	no	no	10^1-10^3 pfu/mL	< 120 min
Mass-based	synthetic	no	no	10^6 pfu/mL	< 60 min
Optical-based	synthetic	no	no	$> 10^3$ pfu/mL	< 60 min
Spectroscopy-based	synthetic	no	no	$> 10^2$ pfu/mL	< 60 min
Particle counting-based	synthetic and water samples	dilutions	< 60 min	$\geq 10^4$ pfu/mL	< 60 min

As seen in the Table 15, most phage detection and quantification methods are either evaluated in synthetic media or in water samples. The reason for this is that most phage detection methods developed, consider phages as safe surrogates for human viral pathogens, which are easy to propagate and to work with in the laboratory. The type of phage used in these studies must be similar to eukaryotic viruses of interest, in terms of shape, size, surface properties and type of nucleic acid, DNA or RNA. For instance, bacteriophage T7 is a good model for adenoviruses as they are morphologically similar and devoid of lipidic envelope. Bacteriophage MS2 is similar in shape, size and type of nucleic acid (RNA) to smallpox (Thomas et al. 2004), waterborne viruses like enteroviruses, caliciviruses, and hepatitis virus A (Shirale et al. 2010), and is frequently used as a model for the aerosol dispersal of airborne pathogens (Usachev et al. 2012). In addition, phages PR772 and Φ6 are good surrogates for influenza A (Deboosere et al. 2012). Next, to detect phages in water samples is of importance in microbial source tracking studies. In water safety, coliphages are used as fecal indicators for drinking or surface water that can be related to the source of contamination as well as to the presence of pathogenic microorganisms. For instance, F^+ specific RNA phages genogroup I and IV are predominantly found in animal waste, while genogroup II and III are related to human waste origin (Wolf et al. 2008). In addition, the F^+ specific RNA genogroup

II bacteriophage have been proposed as a good viral indicator for norovirus detection in shellfish (Hartard et al. 2017). As seen from Table 15, DNA based methods are often used to detect and quantify phages in dairy industry. This is the case because they can detect both lytic and "hidden" lysogenic/temperate phages in the genomes of lactic acid bacteria used in starter cultures. Thus, this type of methods are very helpful and useful for dairy industry in order to prevent economic losses due to partial or complete interruption of fermentations, poor product quality and consistency as well as emergence of spoilage bacteria and pathogens (Muhammed et al. 2017). Regarding sample pretreatments, most of the methods do not require of any pre-treatment step. This is mainly related to the type of sample being analyzed, in most cases synthetic. However, in the case of nucleic acid-based methods, sample pretreatments are often needed as complex matrices such as milk or wastewater can interfere with the assay, by inhibiting its mechanism or by decreasing its sensitivity. The time usually spent on these processes is often less than 2 hours. In terms of LOD and Time of Detection, we can see that cell-based methods together with DNA-based, Electrochemical and Spectroscopy-based methods can achieve high sensitivities with LOD often lower than 10^2 pfu/mL. Concerning the length of the assays (Time of Detection) cell-based methods and immunoassays are in clear disadvantage. In most cases Time of Detection exceeds 3 hours, while other group of methods detects phages in less than 60 min. This difference is somewhat related to the technology used in which fast response times require high-end instrumentation. Methods that can detect and quantify phages with great sensitivity in a short period of time are electrochemical, optical and spectroscopy-based techniques.

Summarizing, the analytical approaches developed in this thesis using *E. coli* and T4 as model organisms follow a time-kill assay format. In both cases phages were detected through monitoring the growth/lysis kinetics of *E. coli* cultures challenged with different concentrations of bacteriophage. In one case phage detection and quantification was achieved by comparing the OD kinetics of control and phage-infected cultures. In the second approach, the same kinetics were monitored with the help of the fluorescent cell viability dye resazurin. Both OD and fluorescence-based approaches are complementary and have similar LOD and Time of Detection ($5x10^1$ pfu/mL

in 3.5h). OD_{600} assay has greater quantification power, and the resazurin assay can detect high phage number faster, in less than 60 min. The working principle of both assays is highly flexible and can be easily adjusted to any specific application by choosing the right phage-host combination whether it be in clean or high complexity samples. Finally, the technology used is relatively simple and well suited for further miniaturization, automatization and high-throughput analysis.

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5. Annex

5.1 First publication article

Fast phage detection and quantification: An optical density-based approach. This work has been published in the journal PLOS ONE.

PLOS ONE

RESEARCH ARTICLE

Fast phage detection and quantification: An optical density-based approach

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Abstract

Since 1959 with the proposal of Double Agar Layer (DAL) method for phage detection and quantification, many sophisticated methods have emerged meanwhile. However, many of them are either too complex/expensive or insensitive to replace routine utilization of DAL method in clinical, environmental and industrial environments. For that purpose, we have explored an alternative method for the detection and quantification of bacteriophages that fulfills the criteria of being rapid, simple and inexpensive. In this paper we have developed a method based on the analysis of optical density kinetics in bacterial cultures exposed to phage-containing samples. Although the decrease in optical density caused by cell lysis was one of the first observable consequences of the effect of viral infection in bacterial cultures, the potential of the method for the assessment of phage abundance has never been fully exploited. In this work we carry out a detailed study of optical density kinetics in phage-infected bacterial cultures, as a function of both, phage abundance and initial concentration of the host organisms. In total, 90 different combinations of bacteria/phage concentrations have been used. The data obtained provide valuable information about sensitivity ranges, duration of the assay, percentages of inhibition and type of lysing behavior for each phage concentration. The method described can detect, as few as 10 phage particles per assay volume after a phage incubation period of 3.5h. The duration of the assay can be shortened to 45min at the expense of losing sensitivity and increasing the limit of detection to 10^8 pfu/ml. Despite using non-sophisticated technology, the method described has shown sensitivity and response time comparable to other high-end methods. The simplicity of the technology and of the analytical steps involved, make the system susceptible of miniaturization and automation for high-throughput applications which can be implemented in routine analysis in many environments.

Citation: Rajnovic D, Muñoz-Berbel X, Mas J (2019) Fast phage detection and quantification: An optical density-based approach. PLoS ONE 14(5): e0216292. https://doi.org/10.1371/journal.pone.0216292

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Introduction

Methods for the detection and quantification of bacteriophages have been available ever since their discovery by Felix d'Herelle in 1917 [1]. These methods, based on the presence of lysis plaques in lawns of host bacteria growing in a double agar layer (DAL), were described in

5.1.1 First publication dataset

S1 Dataset. This dataset contains the optical density (OD) vs time data corresponding to 90 different phage/bacteria combinations used in the paper "Fast phage detection and quantification: an optical density-based approach" by Denis Rajnovic, Xavier Muñoz Berbel and Jordi Mas. Data have been used to build the graphs in Supplemental Figure S1. A subset of the data has been used for Figure 4 of the paper.

5x10^8 cfu/ml																												
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
5.5	0.716	0.863	0.812	0.820	0.832	0.840	0.829	0.881	0.895	0.838	0.897	0.906	0.813	0.834	0.832	0.825	0.846	0.845	0.824	0.845	0.843	0.826	0.847	0.845	0.829	0.850	0.848
11.0	0.776	0.827	0.828	0.830	0.829	0.836	0.868	0.834	0.844	0.855	0.852	0.848	0.848	0.779	0.851	0.860	0.791	0.863	0.859	0.790	0.862	0.861	0.792	0.864	0.864	0.795	0.867
16.5	0.801	0.864	0.849	0.865	0.857	0.858	0.902	0.869	0.872	0.889	0.892	0.874	0.883	0.845	0.877	0.896	0.857	0.889	0.894	0.856	0.888	0.896	0.858	0.890	0.899	0.860	0.893
22.0	0.687	0.807	0.775	0.879	0.880	0.883	0.931	0.897	0.903	0.921	0.922	0.908	0.917	0.886	0.900	0.930	0.898	0.913	0.928	0.897	0.912	0.930	0.899	0.914	0.933	0.902	0.916
27.5	0.586	0.692	0.652	0.878	0.876	0.886	0.950	0.918	0.927	0.942	0.947	0.935	0.940	0.915	0.920	0.953	0.927	0.932	0.951	0.926	0.931	0.953	0.928	0.933	0.956	0.931	0.936
33.0	0.553	0.638	0.595	0.875	0.875	0.883	0.960	0.938	0.941	0.954	0.964	0.949	0.958	0.931	0.932	0.970	0.943	0.944	0.969	0.942	0.943	0.971	0.944	0.945	0.974	0.946	0.948
38.5							0.969	0.947	0.950	0.959	0.975	0.964	0.969	0.944	0.943	0.982	0.956	0.955	0.980	0.955	0.954	0.982	0.957	0.956	0.985	0.960	0.959
44.0	0.538	0.609	0.568	0.882	0.882	0.883	0.975	0.953	0.956	0.974	0.985	0.971	0.978	0.954	0.949	0.990	0.966	0.962	0.989	0.965	0.961	0.991	0.967	0.963	0.994	0.970	0.965
49.5	0.533	0.606	0.567	0.886	0.886	0.888	0.982	0.964	0.963	0.980	0.992	0.978	0.987	0.963	0.961	1.000	0.975	0.974	0.999	0.974	0.973	1.001	0.976	0.975	1.003	0.979	0.977
55.0	0.510	0.602	0.562	0.887	0.891	0.892	0.984	0.971	0.970	0.986	1.000	0.984	0.992	0.972	0.968	1.004	0.985	0.980	1.003	0.983	0.979	1.005	0.985	0.981	1.007	0.988	0.983
66.0	0.412	0.534	0.482	0.889	0.884	0.898	0.977	0.973	0.971	0.982	0.997	0.996	0.987	0.979	0.971	0.999	0.992	0.983	0.998	0.991	0.982	1.000	0.993	0.984	1.003	0.995	0.987
71.5	0.382	0.496	0.435	0.884	0.879	0.890	0.973	0.968	0.968	0.983	0.994	0.995	0.988	0.977	0.971	1.000	0.989	0.983	0.999	0.988	0.982	1.001	0.990	0.984	1.004	0.992	0.987
77.0	0.363	0.471	0.408	0.882	0.876	0.887	0.973	0.967	0.966	0.983	0.993	0.993	0.988	0.978	0.968	1.000	0.990	0.981	0.999	0.989	0.980	1.001	0.991	0.982	1.004	0.994	0.984
92.5	0.335	0.430	0.368	0.873	0.866	0.874	0.968	0.966	0.961	0.982	0.990	0.989	0.987	0.981	0.970	0.999	0.993	0.983	0.998	0.992	0.982	1.000	0.994	0.984	1.003	0.996	0.986
99.0	0.335	0.430	0.368	0.873	0.866	0.874	0.968	0.966	0.961	0.982	0.990	0.989	0.987	0.981	0.970	0.999	0.993	0.983	0.998	0.992	0.982	1.000	0.994	0.984	1.003	0.996	0.986
105.5	0.324	0.420	0.359	0.869	0.860	0.870	0.969	0.965	0.958	0.983	0.992	0.989	0.987	0.980	0.970	0.999	0.993	0.983	0.998	0.992	0.981	1.000	0.994	0.983	1.002	0.996	0.986
112.0	0.309	0.412	0.350	0.865	0.855	0.864	0.967	0.964	0.953	0.986	0.991	0.988	0.990	0.983	0.970	1.002	0.995	0.982	1.001	0.994	0.981	1.003	0.996	0.983	1.005	0.998	0.985
118.5	0.295	0.403	0.340	0.863	0.853	0.860	0.969	0.965	0.952	0.984	0.993	0.988	0.993	0.982	0.969	1.005	0.994	0.982	1.004	0.993	0.980	1.006	0.995	0.982	1.008	0.998	0.985
125.0	0.284	0.394	0.328	0.861	0.850	0.859	0.970	0.965	0.954	0.989	0.994	0.989	0.998	0.984	0.970	1.010	0.996	0.982	1.009	0.995	0.981	1.011	0.997	0.983	1.014	1.000	0.986
131.5	0.276	0.381	0.313	0.862	0.849	0.859	0.968	0.963	0.951	0.995	0.992	0.989	1.003	0.988	0.970	1.015	1.000	0.983	1.014	0.999	0.981	1.016	1.001	0.983	1.019	1.004	0.986
138.0	0.270	0.361	0.297	0.865	0.851	0.860	0.971	0.967	0.954	0.999	0.996	0.990	1.006	0.993	0.975	1.018	1.005	0.987	1.017	1.004	0.986	1.019	1.006	0.988	1.022	1.008	0.991
144.5	0.265	0.346	0.287	0.865	0.851	0.860	0.975	0.970	0.955	1.002	1.000	0.992	1.007	0.995	0.976	1.020	1.007	0.988	1.019	1.006	0.987	1.021	1.008	0.989	1.023	1.011	0.992
151.0	0.262	0.336	0.282	0.865	0.852	0.860	0.976	0.971	0.957	1.005	1.003	0.994	1.009	0.996	0.980	1.022	1.009	0.992	1.021	1.008	0.991	1.023	1.010	0.993	1.025	1.012	0.995
157.5	0.260	0.329	0.277	0.865	0.851	0.860	0.978	0.973	0.959	1.005	1.007	0.997	1.011	0.998	0.981	1.024	1.010	0.994	1.022	1.009	0.993	1.024	1.011	0.995	1.027	1.014	0.997

164.0	**0.259**	**0.321**	**0.272**	**0.864**	**0.851**	**0.860**	**0.979**	**0.974**	**0.959**	**1.006**	**1.007**	**0.998**	**1.012**	**1.000**	**0.982**	**1.024**	**1.012**	**0.994**	**1.023**	**1.011**	**0.993**	**1.025**	**1.013**	**0.995**	**1.027**	**1.016**	**0.997**
180.0	**0.253**	**0.299**	**0.262**	**0.854**	**0.842**	**0.853**	**0.978**	**0.978**	**0.961**	**0.999**	**1.012**	**1.002**	**1.001**	**1.001**	**0.977**	**1.014**	**1.013**	**0.989**	**1.013**	**1.012**	**0.988**	**1.015**	**1.014**	**0.990**	**1.017**	**1.016**	**0.993**
185.5	**0.254**	**0.299**	**0.264**	**0.858**	**0.849**	**0.858**	**0.979**	**0.975**	**0.959**	**0.998**	**1.005**	**1.001**	**1.002**	**0.993**	**0.982**	**1.014**	**1.005**	**0.994**	**1.013**	**1.004**	**0.993**	**1.015**	**1.006**	**0.995**	**1.018**	**1.008**	**0.997**
191.0	**0.253**	**0.298**	**0.263**	**0.863**	**0.845**	**0.858**	**0.975**	**0.970**	**0.959**	**0.999**	**1.005**	**1.000**	**1.007**	**0.995**	**0.986**	**1.019**	**1.008**	**0.998**	**1.018**	**1.006**	**0.997**	**1.020**	**1.008**	**0.999**	**1.023**	**1.011**	**1.002**
196.5	**0.252**	**0.297**	**0.262**	**0.865**	**0.849**	**0.860**	**0.974**	**0.972**	**0.959**	**1.001**	**1.008**	**1.000**	**1.009**	**0.998**	**0.984**	**1.022**	**1.010**	**0.997**	**1.021**	**1.009**	**0.996**	**1.023**	**1.011**	**0.998**	**1.025**	**1.014**	**1.000**
202.0	**0.250**	**0.296**	**0.261**	**0.866**	**0.849**	**0.859**	**0.977**	**0.973**	**0.958**	**1.007**	**1.009**	**1.000**	**1.011**	**1.001**	**0.984**	**1.024**	**1.013**	**0.996**	**1.022**	**1.012**	**0.995**	**1.024**	**1.014**	**0.997**	**1.027**	**1.016**	**1.000**
207.5	**0.248**	**0.294**	**0.260**	**0.866**	**0.848**	**0.859**	**0.976**	**0.975**	**0.958**	**1.007**	**1.009**	**1.001**	**1.012**	**1.002**	**0.984**	**1.025**	**1.014**	**0.997**	**1.023**	**1.013**	**0.995**	**1.025**	**1.015**	**0.997**	**1.028**	**1.018**	**1.000**
213.0	**0.246**	**0.293**	**0.259**	**0.867**	**0.848**	**0.860**	**0.977**	**0.977**	**0.957**	**1.007**	**1.011**	**1.000**	**1.015**	**1.002**	**0.985**	**1.027**	**1.015**	**0.997**	**1.026**	**1.014**	**0.996**	**1.028**	**1.016**	**0.998**	**1.031**	**1.018**	**1.001**
218.5	**0.245**	**0.292**	**0.258**	**0.867**	**0.848**	**0.860**	**0.977**	**0.976**	**0.960**	**1.007**	**1.013**	**1.002**	**1.017**	**1.005**	**0.986**	**1.030**	**1.017**	**0.998**	**1.028**	**1.016**	**0.997**	**1.030**	**1.018**	**0.999**	**1.033**	**1.020**	**1.002**
224.0	**0.244**	**0.291**	**0.256**	**0.868**	**0.848**	**0.860**	**0.978**	**0.977**	**0.960**	**1.011**	**1.012**	**1.004**	**1.019**	**1.005**	**0.987**	**1.031**	**1.018**	**1.000**	**1.030**	**1.016**	**0.999**	**1.032**	**1.018**	**1.001**	**1.034**	**1.021**	**1.003**
229.5	**0.243**	**0.290**	**0.256**	**0.868**	**0.848**	**0.859**	**0.976**	**0.981**	**0.963**	**1.010**	**1.016**	**1.006**	**1.020**	**1.006**	**0.987**	**1.033**	**1.018**	**1.000**	**1.032**	**1.017**	**0.998**	**1.034**	**1.019**	**1.000**	**1.036**	**1.022**	**1.003**

2,5x10^8 cfu/ml																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5.5	**0.435**	**0.467**	**0.450**	**0.548**	**0.493**	**0.506**	**0.498**	**0.504**	**0.485**	**0.490**	**0.519**	**0.519**	**0.488**	**0.511**	**0.491**	**0.493**	**0.516**	**0.496**	**0.497**	**0.520**	**0.500**	**0.503**	**0.526**	**0.506**	**0.508**	**0.531**	**0.511**
11.0	**0.450**	**0.463**	**0.458**	**0.445**	**0.497**	**0.478**	**0.491**	**0.509**	**0.493**	**0.508**	**0.527**	**0.510**	**0.473**	**0.510**	**0.497**	**0.478**	**0.515**	**0.502**	**0.482**	**0.519**	**0.506**	**0.488**	**0.524**	**0.512**	**0.493**	**0.530**	**0.517**
16.5	**0.459**	**0.466**	**0.460**	**0.503**	**0.517**	**0.504**	**0.493**	**0.529**	**0.512**	**0.508**	**0.534**	**0.519**	**0.499**	**0.518**	**0.509**	**0.504**	**0.523**	**0.514**	**0.508**	**0.527**	**0.518**	**0.514**	**0.533**	**0.524**	**0.519**	**0.538**	**0.529**
22.0	**0.377**	**0.379**	**0.378**	**0.518**	**0.526**	**0.517**	**0.527**	**0.553**	**0.542**	**0.519**	**0.554**	**0.547**	**0.533**	**0.548**	**0.538**	**0.538**	**0.553**	**0.543**	**0.542**	**0.557**	**0.547**	**0.547**	**0.563**	**0.553**	**0.552**	**0.568**	**0.558**
27.5	**0.299**	**0.296**	**0.300**	**0.528**	**0.529**	**0.521**	**0.567**	**0.583**	**0.572**	**0.572**	**0.590**	**0.585**	**0.574**	**0.584**	**0.577**	**0.580**	**0.589**	**0.582**	**0.583**	**0.593**	**0.586**	**0.589**	**0.598**	**0.592**	**0.594**	**0.603**	**0.597**
33.0	**0.263**	**0.258**	**0.260**	**0.534**	**0.528**	**0.517**	**0.598**	**0.616**	**0.603**	**0.617**	**0.630**	**0.623**	**0.609**	**0.622**	**0.611**	**0.614**	**0.627**	**0.616**	**0.618**	**0.631**	**0.620**	**0.623**	**0.636**	**0.625**	**0.628**	**0.641**	**0.630**
38.5							**0.624**	**0.645**	**0.631**	**0.648**	**0.660**	**0.656**	**0.640**	**0.654**	**0.642**	**0.645**	**0.659**	**0.647**	**0.648**	**0.663**	**0.651**	**0.654**	**0.669**	**0.656**	**0.659**	**0.674**	**0.661**
44.0	**0.244**	**0.237**	**0.237**	**0.558**	**0.549**	**0.540**	**0.653**	**0.673**	**0.660**	**0.673**	**0.687**	**0.683**	**0.663**	**0.681**	**0.671**	**0.668**	**0.686**	**0.676**	**0.672**	**0.690**	**0.679**	**0.678**	**0.696**	**0.685**	**0.683**	**0.701**	**0.690**
49.5	**0.228**	**0.221**	**0.222**	**0.566**	**0.560**	**0.550**	**0.670**	**0.688**	**0.678**	**0.690**	**0.708**	**0.699**	**0.683**	**0.700**	**0.686**	**0.688**	**0.705**	**0.691**	**0.692**	**0.709**	**0.695**	**0.698**	**0.715**	**0.700**	**0.703**	**0.720**	**0.705**
55.0	**0.200**	**0.195**	**0.195**	**0.566**	**0.561**	**0.553**	**0.682**	**0.697**	**0.691**	**0.702**	**0.723**	**0.717**	**0.695**	**0.712**	**0.703**	**0.700**	**0.717**	**0.708**	**0.704**	**0.721**	**0.712**	**0.709**	**0.726**	**0.718**	**0.714**	**0.731**	**0.723**
66.0	**0.175**	**0.174**	**0.171**	**0.517**	**0.507**	**0.509**	**0.704**	**0.715**	**0.713**	**0.730**	**0.742**	**0.740**	**0.727**	**0.730**	**0.727**	**0.732**	**0.735**	**0.733**	**0.736**	**0.739**	**0.736**	**0.741**	**0.744**	**0.742**	**0.746**	**0.749**	**0.747**
71.5	**0.173**	**0.171**	**0.168**	**0.491**	**0.480**	**0.481**	**0.706**	**0.717**	**0.714**	**0.742**	**0.751**	**0.750**	**0.737**	**0.742**	**0.736**	**0.742**	**0.747**	**0.742**	**0.746**	**0.751**	**0.745**	**0.752**	**0.757**	**0.751**	**0.757**	**0.762**	**0.756**
77.0	**0.170**	**0.170**	**0.166**	**0.474**	**0.462**	**0.463**	**0.705**	**0.715**	**0.714**	**0.744**	**0.754**	**0.750**	**0.741**	**0.744**	**0.739**	**0.746**	**0.750**	**0.744**	**0.750**	**0.753**	**0.748**	**0.756**	**0.759**	**0.753**	**0.761**	**0.764**	**0.758**
92.5	**0.167**	**0.168**	**0.163**	**0.435**	**0.423**	**0.424**	**0.709**	**0.718**	**0.716**	**0.756**	**0.765**	**0.759**	**0.754**	**0.757**	**0.753**	**0.759**	**0.762**	**0.758**	**0.763**	**0.766**	**0.762**	**0.769**	**0.772**	**0.768**	**0.774**	**0.777**	**0.773**
99.0	**0.167**	**0.168**	**0.163**	**0.435**	**0.423**	**0.424**	**0.709**	**0.718**	**0.716**	**0.756**	**0.765**	**0.759**	**0.754**	**0.757**	**0.753**	**0.759**	**0.762**	**0.758**	**0.763**	**0.766**	**0.762**	**0.769**	**0.772**	**0.768**	**0.774**	**0.777**	**0.773**
105.5	**0.165**	**0.167**	**0.162**	**0.416**	**0.402**	**0.405**	**0.706**	**0.716**	**0.714**	**0.756**	**0.764**	**0.762**	**0.754**	**0.760**	**0.755**	**0.759**	**0.765**	**0.760**	**0.763**	**0.769**	**0.764**	**0.768**	**0.774**	**0.769**	**0.773**	**0.779**	**0.774**
112.0	**0.164**	**0.166**	**0.162**	**0.381**	**0.367**	**0.371**	**0.706**	**0.718**	**0.714**	**0.760**	**0.765**	**0.764**	**0.758**	**0.763**	**0.757**	**0.763**	**0.768**	**0.762**	**0.767**	**0.772**	**0.766**	**0.772**	**0.778**	**0.771**	**0.777**	**0.783**	**0.776**

118.5	**0.164**	**0.166**	**0.161**	**0.347**	**0.327**	**0.335**	**0.709**	**0.720**	**0.715**	**0.761**	**0.768**	**0.768**	**0.759**	**0.765**	**0.760**	**0.764**	**0.770**	**0.765**	**0.768**	**0.774**	**0.769**	**0.774**	**0.779**	**0.775**	**0.779**	**0.785**	**0.780**
125.0	**0.163**	**0.166**	**0.160**	**0.316**	**0.292**	**0.303**	**0.706**	**0.718**	**0.717**	**0.760**	**0.768**	**0.766**	**0.760**	**0.765**	**0.762**	**0.765**	**0.770**	**0.767**	**0.769**	**0.774**	**0.771**	**0.774**	**0.779**	**0.776**	**0.779**	**0.784**	**0.781**
131.5	**0.162**	**0.165**	**0.160**	**0.291**	**0.266**	**0.279**	**0.705**	**0.716**	**0.716**	**0.761**	**0.769**	**0.766**	**0.761**	**0.766**	**0.764**	**0.766**	**0.771**	**0.769**	**0.770**	**0.775**	**0.773**	**0.775**	**0.780**	**0.778**	**0.780**	**0.785**	**0.784**
138.0	**0.161**	**0.165**	**0.159**	**0.273**	**0.246**	**0.260**	**0.707**	**0.717**	**0.716**	**0.764**	**0.768**	**0.766**	**0.764**	**0.766**	**0.767**	**0.769**	**0.771**	**0.772**	**0.773**	**0.775**	**0.776**	**0.778**	**0.781**	**0.781**	**0.783**	**0.786**	**0.787**
144.5	**0.161**	**0.164**	**0.159**	**0.259**	**0.231**	**0.246**	**0.707**	**0.716**	**0.716**	**0.766**	**0.769**	**0.766**	**0.764**	**0.768**	**0.769**	**0.769**	**0.773**	**0.774**	**0.773**	**0.777**	**0.778**	**0.779**	**0.783**	**0.784**	**0.784**	**0.788**	**0.789**
151.0	**0.161**	**0.164**	**0.158**	**0.248**	**0.220**	**0.234**	**0.705**	**0.715**	**0.714**	**0.768**	**0.770**	**0.768**	**0.767**	**0.771**	**0.772**	**0.772**	**0.776**	**0.777**	**0.776**	**0.780**	**0.781**	**0.782**	**0.785**	**0.787**	**0.787**	**0.790**	**0.792**
157.5	**0.160**	**0.164**	**0.158**	**0.241**	**0.212**	**0.226**	**0.704**	**0.716**	**0.715**	**0.770**	**0.772**	**0.771**	**0.769**	**0.771**	**0.775**	**0.774**	**0.776**	**0.780**	**0.778**	**0.780**	**0.784**	**0.783**	**0.786**	**0.790**	**0.788**	**0.791**	**0.795**
164.0	**0.160**	**0.163**	**0.158**	**0.236**	**0.207**	**0.221**	**0.704**	**0.714**	**0.716**	**0.773**	**0.775**	**0.773**	**0.772**	**0.775**	**0.778**	**0.777**	**0.780**	**0.783**	**0.781**	**0.784**	**0.787**	**0.786**	**0.789**	**0.792**	**0.791**	**0.794**	**0.797**
180.0	**0.159**	**0.162**	**0.156**	**0.230**	**0.198**	**0.215**	**0.701**	**0.716**	**0.711**	**0.784**	**0.776**	**0.781**	**0.782**	**0.779**	**0.782**	**0.787**	**0.784**	**0.788**	**0.790**	**0.788**	**0.791**	**0.796**	**0.794**	**0.797**	**0.801**	**0.799**	**0.802**
185.5	**0.160**	**0.163**	**0.158**	**0.228**	**0.195**	**0.214**	**0.694**	**0.708**	**0.711**	**0.776**	**0.778**	**0.777**	**0.778**	**0.784**	**0.786**	**0.783**	**0.789**	**0.791**	**0.787**	**0.793**	**0.795**	**0.792**	**0.799**	**0.801**	**0.797**	**0.804**	**0.806**
191.0	**0.160**	**0.163**	**0.157**	**0.227**	**0.194**	**0.213**	**0.699**	**0.713**	**0.712**	**0.785**	**0.779**	**0.780**	**0.783**	**0.785**	**0.792**	**0.788**	**0.790**	**0.797**	**0.792**	**0.794**	**0.801**	**0.797**	**0.799**	**0.806**	**0.802**	**0.804**	**0.811**
196.5	**0.159**	**0.163**	**0.157**	**0.226**	**0.192**	**0.211**	**0.700**	**0.713**	**0.713**	**0.785**	**0.782**	**0.784**	**0.783**	**0.786**	**0.794**	**0.788**	**0.791**	**0.799**	**0.792**	**0.795**	**0.803**	**0.797**	**0.801**	**0.809**	**0.802**	**0.806**	**0.814**
202.0	**0.159**	**0.162**	**0.157**	**0.225**	**0.191**	**0.210**	**0.700**	**0.715**	**0.711**	**0.785**	**0.786**	**0.784**	**0.785**	**0.787**	**0.796**	**0.790**	**0.792**	**0.801**	**0.794**	**0.796**	**0.805**	**0.800**	**0.801**	**0.811**	**0.805**	**0.807**	**0.816**
207.5	**0.159**	**0.162**	**0.156**	**0.223**	**0.189**	**0.207**	**0.699**	**0.715**	**0.710**	**0.787**	**0.786**	**0.786**	**0.786**	**0.786**	**0.798**	**0.791**	**0.791**	**0.803**	**0.795**	**0.795**	**0.807**	**0.800**	**0.801**	**0.813**	**0.805**	**0.806**	**0.818**
213.0	**0.159**	**0.162**	**0.157**	**0.220**	**0.187**	**0.204**	**0.698**	**0.714**	**0.708**	**0.787**	**0.787**	**0.787**	**0.787**	**0.791**	**0.801**	**0.792**	**0.796**	**0.806**	**0.795**	**0.800**	**0.810**	**0.801**	**0.805**	**0.816**	**0.806**	**0.810**	**0.821**
218.5	**0.159**	**0.162**	**0.157**	**0.218**	**0.186**	**0.203**	**0.697**	**0.713**	**0.708**	**0.791**	**0.793**	**0.789**	**0.790**	**0.795**	**0.805**	**0.795**	**0.800**	**0.810**	**0.799**	**0.804**	**0.814**	**0.804**	**0.810**	**0.819**	**0.809**	**0.815**	**0.825**
224.0	**0.159**	**0.163**	**0.157**	**0.216**	**0.185**	**0.201**	**0.694**	**0.713**	**0.706**	**0.791**	**0.793**	**0.789**	**0.792**	**0.796**	**0.808**	**0.797**	**0.801**	**0.813**	**0.801**	**0.805**	**0.817**	**0.807**	**0.811**	**0.823**	**0.812**	**0.816**	**0.828**
229.5	**0.159**	**0.162**	**0.156**	**0.214**	**0.184**	**0.200**	**0.690**	**0.710**	**0.704**	**0.795**	**0.797**	**0.793**	**0.791**	**0.798**	**0.811**	**0.796**	**0.803**	**0.816**	**0.800**	**0.807**	**0.820**	**0.806**	**0.813**	**0.825**	**0.811**	**0.818**	**0.830**

10^8 cfu/ml																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5.0	**0.251**	**0.257**	**0.255**	**0.264**	**0.264**	**0.273**	**0.270**	**0.267**	**0.274**	**0.255**	**0.274**	**0.268**	**0.282**	**0.289**	**0.287**	**0.258**	**0.288**	**0.278**	**0.279**	**0.287**	**0.283**	**0.282**	**0.277**	**0.283**	**0.284**	**0.279**	**0.285**
10.0	**0.256**	**0.258**	**0.255**	**0.265**	**0.266**	**0.267**	**0.269**	**0.267**	**0.274**	**0.256**	**0.273**	**0.274**	**0.281**	**0.294**	**0.281**	**0.256**	**0.280**	**0.278**	**0.279**	**0.284**	**0.280**	**0.289**	**0.287**	**0.284**	**0.291**	**0.289**	**0.286**
15.0	**0.255**	**0.256**	**0.254**	**0.268**	**0.269**	**0.268**	**0.276**	**0.275**	**0.282**	**0.263**	**0.280**	**0.283**	**0.289**	**0.300**	**0.291**	**0.263**	**0.284**	**0.287**	**0.288**	**0.294**	**0.291**	**0.300**	**0.299**	**0.300**	**0.302**	**0.301**	**0.302**
20.0	**0.231**	**0.235**	**0.232**	**0.274**	**0.274**	**0.275**	**0.288**	**0.288**	**0.294**	**0.274**	**0.287**	**0.293**	**0.302**	**0.313**	**0.300**	**0.276**	**0.293**	**0.297**	**0.305**	**0.309**	**0.305**	**0.322**	**0.318**	**0.318**	**0.324**	**0.320**	**0.320**
25.0	**0.165**	**0.169**	**0.165**	**0.262**	**0.263**	**0.265**	**0.304**	**0.304**	**0.308**	**0.290**	**0.305**	**0.308**	**0.319**	**0.331**	**0.317**	**0.290**	**0.313**	**0.318**	**0.325**	**0.328**	**0.326**	**0.345**	**0.339**	**0.342**	**0.347**	**0.341**	**0.344**
30.0	**0.139**	**0.142**	**0.141**	**0.245**	**0.246**	**0.245**	**0.321**	**0.319**	**0.327**	**0.308**	**0.327**	**0.331**	**0.343**	**0.352**	**0.341**	**0.312**	**0.337**	**0.341**	**0.350**	**0.353**	**0.352**	**0.377**	**0.373**	**0.373**	**0.379**	**0.375**	**0.375**
35.0							**0.346**	**0.344**	**0.349**	**0.335**	**0.354**	**0.356**	**0.371**	**0.378**	**0.367**	**0.339**	**0.368**	**0.370**	**0.384**	**0.385**	**0.384**	**0.416**	**0.410**	**0.409**	**0.418**	**0.412**	**0.411**
40.0	**0.123**	**0.125**	**0.125**	**0.234**	**0.233**	**0.235**	**0.374**	**0.374**	**0.378**	**0.367**	**0.384**	**0.390**	**0.406**	**0.407**	**0.397**	**0.370**	**0.403**	**0.405**	**0.422**	**0.424**	**0.418**	**0.452**	**0.445**	**0.445**	**0.454**	**0.447**	**0.447**
45.0	**0.120**	**0.122**	**0.123**	**0.238**	**0.235**	**0.237**	**0.402**	**0.400**	**0.407**	**0.398**	**0.415**	**0.424**	**0.439**	**0.440**	**0.434**	**0.403**	**0.436**	**0.442**	**0.458**	**0.463**	**0.457**	**0.487**	**0.481**	**0.482**	**0.489**	**0.483**	**0.484**
50.0	**0.119**	**0.120**	**0.121**	**0.240**	**0.238**	**0.240**	**0.433**	**0.432**	**0.435**	**0.434**	**0.449**	**0.460**	**0.481**	**0.478**	**0.469**	**0.441**	**0.473**	**0.477**	**0.497**	**0.497**	**0.496**	**0.516**	**0.510**	**0.511**	**0.518**	**0.512**	**0.513**

55.0	**0.118**	**0.119**	**0.120**	**0.232**	**0.231**	**0.233**	**0.458**	**0.455**	**0.462**	**0.464**	**0.478**	**0.489**	**0.511**	**0.504**	**0.500**	**0.473**	**0.503**	**0.507**	**0.525**	**0.524**	**0.521**	**0.543**	**0.533**	**0.536**	**0.545**	**0.535**	**0.538**
60.0	**0.116**	**0.118**	**0.119**	**0.215**	**0.215**	**0.218**	**0.473**	**0.472**	**0.479**	**0.486**	**0.500**	**0.515**	**0.532**	**0.527**	**0.526**	**0.496**	**0.526**	**0.531**	**0.552**	**0.553**	**0.550**	**0.565**	**0.554**	**0.562**	**0.567**	**0.556**	**0.564**
65.0	**0.116**	**0.118**	**0.118**	**0.199**	**0.198**	**0.202**	**0.485**	**0.485**	**0.490**	**0.513**	**0.523**	**0.539**	**0.560**	**0.549**	**0.549**	**0.525**	**0.549**	**0.558**	**0.576**	**0.577**	**0.576**	**0.578**	**0.566**	**0.578**	**0.580**	**0.568**	**0.580**
70.0	**0.116**	**0.117**	**0.117**	**0.187**	**0.185**	**0.188**	**0.497**	**0.495**	**0.502**	**0.535**	**0.541**	**0.563**	**0.583**	**0.570**	**0.575**	**0.549**	**0.571**	**0.581**	**0.596**	**0.595**	**0.595**	**0.593**	**0.581**	**0.590**	**0.595**	**0.583**	**0.592**
75.0	**0.115**	**0.117**	**0.117**	**0.178**	**0.176**	**0.179**	**0.501**	**0.501**	**0.507**	**0.550**	**0.552**	**0.574**	**0.597**	**0.582**	**0.589**	**0.565**	**0.583**	**0.595**	**0.607**	**0.606**	**0.607**	**0.600**	**0.590**	**0.602**	**0.602**	**0.592**	**0.604**
80.0	**0.115**	**0.117**	**0.117**	**0.168**	**0.167**	**0.169**	**0.506**	**0.508**	**0.512**	**0.562**	**0.564**	**0.586**	**0.611**	**0.594**	**0.603**	**0.580**	**0.598**	**0.609**	**0.621**	**0.620**	**0.621**	**0.611**	**0.596**	**0.613**	**0.613**	**0.598**	**0.615**
85.0	**0.114**	**0.117**	**0.117**	**0.154**	**0.153**	**0.156**	**0.509**	**0.508**	**0.515**	**0.573**	**0.573**	**0.598**	**0.623**	**0.604**	**0.615**	**0.593**	**0.607**	**0.620**	**0.632**	**0.632**	**0.631**	**0.619**	**0.607**	**0.622**	**0.621**	**0.609**	**0.624**
90.0	**0.114**	**0.117**	**0.117**	**0.143**	**0.142**	**0.144**	**0.504**	**0.506**	**0.511**	**0.580**	**0.579**	**0.604**	**0.632**	**0.614**	**0.624**	**0.605**	**0.614**	**0.629**	**0.639**	**0.640**	**0.640**	**0.625**	**0.613**	**0.629**	**0.627**	**0.615**	**0.631**
95.0	**0.114**	**0.116**	**0.117**	**0.137**	**0.136**	**0.137**	**0.491**	**0.493**	**0.500**	**0.583**	**0.584**	**0.607**	**0.641**	**0.621**	**0.633**	**0.612**	**0.623**	**0.637**	**0.647**	**0.648**	**0.649**	**0.632**	**0.616**	**0.636**	**0.634**	**0.618**	**0.638**
100.0	**0.115**	**0.117**	**0.117**	**0.135**	**0.133**	**0.134**	**0.469**	**0.475**	**0.481**	**0.588**	**0.590**	**0.610**	**0.648**	**0.628**	**0.644**	**0.617**	**0.629**	**0.646**	**0.657**	**0.658**	**0.657**	**0.640**	**0.623**	**0.639**	**0.642**	**0.625**	**0.641**
105.0	**0.114**	**0.116**	**0.116**	**0.133**	**0.132**	**0.132**	**0.447**	**0.453**	**0.458**	**0.589**	**0.594**	**0.613**	**0.655**	**0.634**	**0.646**	**0.628**	**0.637**	**0.652**	**0.664**	**0.664**	**0.663**	**0.643**	**0.627**	**0.647**	**0.645**	**0.629**	**0.649**
110.0	**0.114**	**0.116**	**0.117**	**0.132**	**0.130**	**0.132**	**0.422**	**0.430**	**0.432**	**0.588**	**0.595**	**0.615**	**0.661**	**0.641**	**0.655**	**0.634**	**0.643**	**0.659**	**0.670**	**0.671**	**0.670**	**0.648**	**0.628**	**0.649**	**0.650**	**0.630**	**0.652**
115.0	**0.114**	**0.116**	**0.117**	**0.130**	**0.129**	**0.130**	**0.397**	**0.407**	**0.408**	**0.590**	**0.600**	**0.614**	**0.667**	**0.647**	**0.661**	**0.642**	**0.648**	**0.666**	**0.673**	**0.674**	**0.673**	**0.654**	**0.631**	**0.656**	**0.656**	**0.633**	**0.658**
120.0	**0.114**	**0.116**	**0.117**	**0.130**	**0.128**	**0.129**	**0.369**	**0.380**	**0.379**	**0.587**	**0.599**	**0.615**	**0.665**	**0.648**	**0.666**	**0.649**	**0.652**	**0.669**	**0.676**	**0.680**	**0.678**	**0.652**	**0.635**	**0.657**	**0.654**	**0.637**	**0.659**
125.0	**0.114**	**0.116**	**0.117**	**0.129**	**0.127**	**0.129**	**0.336**	**0.349**	**0.348**	**0.582**	**0.598**	**0.611**	**0.669**	**0.650**	**0.667**	**0.648**	**0.655**	**0.676**	**0.678**	**0.682**	**0.681**	**0.652**	**0.640**	**0.657**	**0.654**	**0.642**	**0.659**
130.0	**0.114**	**0.115**	**0.116**	**0.128**	**0.126**	**0.127**	**0.301**	**0.315**	**0.311**	**0.575**	**0.597**	**0.610**	**0.674**	**0.654**	**0.672**	**0.656**	**0.659**	**0.680**	**0.680**	**0.685**	**0.685**	**0.655**	**0.642**	**0.661**	**0.657**	**0.644**	**0.663**
135.0	**0.114**	**0.116**	**0.116**	**0.127**	**0.126**	**0.126**	**0.267**	**0.281**	**0.276**	**0.569**	**0.593**	**0.606**	**0.673**	**0.657**	**0.673**	**0.657**	**0.663**	**0.679**	**0.686**	**0.688**	**0.687**	**0.656**	**0.637**	**0.658**	**0.658**	**0.639**	**0.660**
140.0	**0.113**	**0.116**	**0.116**	**0.127**	**0.125**	**0.125**	**0.239**	**0.252**	**0.246**	**0.564**	**0.590**	**0.604**	**0.674**	**0.659**	**0.675**	**0.659**	**0.666**	**0.682**	**0.687**	**0.688**	**0.687**	**0.655**	**0.641**	**0.660**	**0.657**	**0.643**	**0.662**
145.0	**0.113**	**0.116**	**0.116**	**0.126**	**0.124**	**0.125**	**0.218**	**0.228**	**0.221**	**0.552**	**0.586**	**0.594**	**0.676**	**0.659**	**0.679**	**0.661**	**0.666**	**0.682**	**0.688**	**0.690**	**0.688**	**0.659**	**0.641**	**0.664**	**0.661**	**0.643**	**0.666**
150.0	**0.113**	**0.116**	**0.117**	**0.125**	**0.124**	**0.125**	**0.201**	**0.210**	**0.203**	**0.546**	**0.581**	**0.591**	**0.677**	**0.660**	**0.678**	**0.665**	**0.673**	**0.686**	**0.690**	**0.693**	**0.693**	**0.659**	**0.641**	**0.665**	**0.661**	**0.643**	**0.667**
155.0	**0.113**	**0.115**	**0.116**	**0.125**	**0.123**	**0.124**	**0.190**	**0.198**	**0.189**	**0.536**	**0.577**	**0.585**	**0.677**	**0.661**	**0.680**	**0.667**	**0.670**	**0.686**	**0.690**	**0.692**	**0.692**	**0.656**	**0.647**	**0.664**	**0.658**	**0.649**	**0.666**
160.0	**0.112**	**0.115**	**0.115**	**0.124**	**0.123**	**0.124**	**0.181**	**0.188**	**0.180**	**0.527**	**0.572**	**0.577**	**0.679**	**0.663**	**0.681**	**0.671**	**0.673**	**0.690**	**0.693**	**0.695**	**0.693**	**0.663**	**0.652**	**0.667**	**0.665**	**0.654**	**0.669**
165.0	**0.113**	**0.115**	**0.115**	**0.124**	**0.123**	**0.123**	**0.174**	**0.181**	**0.173**	**0.516**	**0.566**	**0.568**	**0.677**	**0.665**	**0.681**	**0.672**	**0.675**	**0.692**	**0.696**	**0.698**	**0.696**	**0.661**	**0.648**	**0.670**	**0.663**	**0.650**	**0.672**
170.0	**0.112**	**0.115**	**0.115**	**0.123**	**0.122**	**0.123**	**0.169**	**0.176**	**0.166**	**0.503**	**0.559**	**0.560**	**0.679**	**0.664**	**0.681**	**0.674**	**0.678**	**0.693**	**0.700**	**0.701**	**0.699**	**0.663**	**0.655**	**0.671**	**0.665**	**0.657**	**0.673**
175.0	**0.113**	**0.115**	**0.116**	**0.123**	**0.123**	**0.123**	**0.164**	**0.171**	**0.162**	**0.492**	**0.552**	**0.552**	**0.679**	**0.665**	**0.681**	**0.674**	**0.676**	**0.696**	**0.701**	**0.702**	**0.701**	**0.667**	**0.658**	**0.675**	**0.669**	**0.660**	**0.677**
180.0	**0.112**	**0.115**	**0.115**	**0.123**	**0.122**	**0.123**	**0.160**	**0.166**	**0.157**	**0.478**	**0.544**	**0.543**	**0.679**	**0.667**	**0.681**	**0.676**	**0.680**	**0.699**	**0.703**	**0.704**	**0.703**	**0.671**	**0.660**	**0.676**	**0.673**	**0.662**	**0.678**
185.0	**0.112**	**0.114**	**0.115**	**0.122**	**0.122**	**0.122**	**0.156**	**0.162**	**0.153**	**0.465**	**0.535**	**0.531**	**0.680**	**0.668**	**0.681**	**0.679**	**0.681**	**0.700**	**0.704**	**0.707**	**0.706**	**0.670**	**0.662**	**0.676**	**0.672**	**0.664**	**0.678**
190.0	**0.112**	**0.114**	**0.116**	**0.122**	**0.122**	**0.122**	**0.152**	**0.159**	**0.150**	**0.451**	**0.526**	**0.522**	**0.682**	**0.669**	**0.682**	**0.679**	**0.682**	**0.703**	**0.709**	**0.711**	**0.708**	**0.675**	**0.667**	**0.682**	**0.677**	**0.669**	**0.684**
195.0	**0.112**	**0.115**	**0.116**	**0.121**	**0.122**	**0.122**	**0.149**	**0.155**	**0.147**	**0.438**	**0.515**	**0.511**	**0.682**	**0.671**	**0.683**	**0.681**	**0.685**	**0.706**	**0.711**	**0.715**	**0.711**	**0.678**	**0.671**	**0.683**	**0.680**	**0.673**	**0.685**
200.0	**0.113**	**0.115**	**0.115**	**0.122**	**0.121**	**0.121**	**0.147**	**0.152**	**0.145**	**0.423**	**0.504**	**0.497**	**0.683**	**0.673**	**0.681**	**0.685**	**0.688**	**0.708**	**0.714**	**0.715**	**0.713**	**0.683**	**0.674**	**0.688**	**0.685**	**0.677**	**0.690**
205.0	**0.113**	**0.115**	**0.116**	**0.121**	**0.121**	**0.121**	**0.144**	**0.149**	**0.142**	**0.409**	**0.493**	**0.486**	**0.682**	**0.673**	**0.679**	**0.685**	**0.688**	**0.711**	**0.718**	**0.720**	**0.717**	**0.687**	**0.678**	**0.691**	**0.689**	**0.680**	**0.693**
210.0	**0.113**	**0.115**	**0.116**	**0.121**	**0.121**	**0.121**	**0.143**	**0.147**	**0.141**	**0.395**	**0.480**	**0.475**	**0.682**	**0.674**	**0.683**	**0.688**	**0.691**	**0.712**	**0.719**	**0.722**	**0.720**	**0.689**	**0.681**	**0.695**	**0.691**	**0.683**	**0.697**
215.0	**0.113**	**0.115**	**0.116**	**0.121**	**0.121**	**0.121**	**0.141**	**0.145**	**0.140**	**0.380**	**0.467**	**0.460**	**0.683**	**0.674**	**0.682**	**0.688**	**0.696**	**0.716**	**0.723**	**0.727**	**0.725**	**0.691**	**0.681**	**0.697**	**0.693**	**0.683**	**0.699**
220.0	**0.113**	**0.115**	**0.116**	**0.120**	**0.120**	**0.121**	**0.140**	**0.142**	**0.139**	**0.367**	**0.453**	**0.448**	**0.684**	**0.674**	**0.680**	**0.689**	**0.697**	**0.717**	**0.726**	**0.730**	**0.727**	**0.695**	**0.683**	**0.699**	**0.697**	**0.685**	**0.701**

225.0	0.113	0.115	0.117	0.121	0.121	0.121	0.139	0.141	0.139	0.355	0.438	0.436	0.680	0.677	0.683	0.691	0.700	0.720	0.728	0.734	0.730	0.697	0.685	0.702	0.699	0.687	0.704
230.0	0.113	0.115	0.116	0.120	0.120	0.121	0.138	0.140	0.138	0.342	0.421	0.423	0.683	0.677	0.681	0.694	0.700	0.724	0.734	0.737	0.735	0.697	0.695	0.706	0.699	0.697	0.708
235.0	0.113	0.115	0.117	0.120	0.121	0.121	0.138	0.139	0.137	0.330	0.402	0.407	0.681	0.676	0.678	0.696	0.705	0.726	0.737	0.741	0.737	0.702	0.697	0.709	0.704	0.699	0.711
240.0	0.113	0.115	0.117	0.120	0.120	0.120	0.138	0.138	0.137	0.320	0.384	0.393	0.679	0.678	0.678	0.698	0.706	0.729	0.738	0.743	0.740	0.705	0.699	0.711	0.707	0.701	0.713
245.0	0.114	0.117	0.118	0.120	0.120	0.121	0.138	0.137	0.138	0.313	0.370	0.376	0.676	0.677	0.675	0.701	0.706	0.731	0.742	0.745	0.742	0.707	0.702	0.713	0.709	0.704	0.715
250.0	0.113	0.116	0.117	0.120	0.120	0.120	0.137	0.137	0.138	0.309	0.360	0.362	0.676	0.675	0.675	0.700	0.712	0.736	0.742	0.748	0.745	0.710	0.704	0.716	0.712	0.706	0.718
255.0	0.114	0.116	0.117	0.120	0.120	0.120	0.137	0.137	0.137	0.308	0.355	0.352	0.673	0.674	0.673	0.699	0.713	0.735	0.745	0.750	0.748	0.712	0.703	0.718	0.714	0.705	0.720

5x10^7 cfu/ml																										
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
3.5	0.172	0.172	0.184	0.173	0.174	0.181	0.170	0.171	0.175	0.173	0.173	0.176	0.181	0.199	0.211	0.190	0.183	0.193	0.183	0.181	0.197	0.166	0.170	0.178	0.175	0.179	0.187
14.0	0.172	0.174	0.182	0.176	0.177	0.184	0.174	0.173	0.181	0.178	0.177	0.182	0.183	0.201	0.218	0.194	0.186	0.198	0.187	0.185	0.200	0.172	0.178	0.187	0.181	0.187	0.196
24.5	0.152	0.148	0.146	0.181	0.181	0.186	0.185	0.185	0.195	0.188	0.189	0.197	0.196	0.211	0.232	0.205	0.198	0.215	0.203	0.205	0.217	0.191	0.202	0.210	0.200	0.211	0.218
31.5	0.116	0.117	0.124	0.161	0.159	0.160	0.197	0.198	0.208	0.205	0.208	0.218	0.213	0.233	0.251	0.222	0.214	0.237	0.224	0.226	0.241	0.217	0.227	0.242	0.225	0.235	0.251
42.0	0.110	0.112	0.117	0.147	0.147	0.152	0.215	0.219	0.231	0.231	0.235	0.251	0.241	0.261	0.286	0.253	0.242	0.269	0.260	0.260	0.273	0.254	0.268	0.282	0.263	0.277	0.290
52.5	0.108	0.110	0.116	0.145	0.146	0.150	0.245	0.251	0.268	0.270	0.275	0.296	0.281	0.302	0.332	0.292	0.282	0.317	0.303	0.305	0.321	0.303	0.315	0.334	0.312	0.324	0.343
59.5							0.299	0.321	0.310	0.304	0.343	0.336	0.319	0.360	0.365	0.328	0.339	0.357	0.351	0.372	0.362	0.352	0.359	0.381	0.361	0.367	0.390
70.0	0.112	0.113	0.120	0.137	0.138	0.142	0.297	0.310	0.328	0.344	0.356	0.378	0.361	0.383	0.417	0.374	0.365	0.404	0.388	0.395	0.408	0.399	0.409	0.428	0.408	0.417	0.437
80.5	0.112	0.113	0.120	0.130	0.132	0.134	0.323	0.338	0.350	0.389	0.399	0.420	0.407	0.427	0.460	0.421	0.411	0.448	0.437	0.442	0.455	0.449	0.459	0.480	0.458	0.468	0.489
94.5	0.111	0.111	0.118	0.121	0.123	0.125	0.340	0.348	0.354	0.437	0.450	0.467	0.466	0.487	0.524	0.481	0.470	0.513	0.507	0.506	0.517	0.512	0.516	0.533	0.521	0.525	0.542
105.0	0.110	0.112	0.118	0.115	0.118	0.120	0.324	0.327	0.317	0.467	0.476	0.490	0.505	0.527	0.557	0.520	0.510	0.547	0.541	0.544	0.551	0.544	0.547	0.564	0.553	0.556	0.573
115.5	0.109	0.111	0.117	0.112	0.116	0.118	0.261	0.255	0.221	0.475	0.481	0.489	0.534	0.558	0.582	0.553	0.542	0.575	0.570	0.574	0.580	0.573	0.575	0.590	0.582	0.584	0.599
126.0	0.109	0.110	0.117	0.111	0.115	0.118	0.181	0.173	0.154	0.465	0.473	0.467	0.559	0.580	0.603	0.581	0.570	0.602	0.598	0.600	0.603	0.593	0.595	0.607	0.602	0.604	0.615
129.5	0.109	0.110	0.117	0.112	0.115	0.117	0.151	0.146	0.139	0.458	0.455	0.436	0.569	0.591	0.609	0.594	0.580	0.614	0.613	0.612	0.616	0.604	0.602	0.613	0.613	0.611	0.622
143.5	0.108	0.110	0.117	0.110	0.114	0.117	0.128	0.127	0.128	0.404	0.389	0.355	0.577	0.591	0.596	0.611	0.601	0.621	0.630	0.627	0.629	0.616	0.618	0.629	0.625	0.627	0.638
150.5	0.108	0.109	0.117	0.109	0.113	0.116	0.122	0.124	0.127	0.343	0.327	0.291	0.570	0.581	0.586	0.615	0.604	0.625	0.633	0.629	0.634	0.621	0.620	0.625	0.630	0.628	0.634
154.0	0.108	0.110	0.117	0.109	0.113	0.116	0.121	0.121	0.124	0.311	0.294	0.261	0.566	0.574	0.576	0.620	0.609	0.626	0.639	0.635	0.638	0.625	0.624	0.632	0.634	0.633	0.641
164.5	0.108	0.110	0.117	0.109	0.113	0.115	0.119	0.119	0.123	0.236	0.227	0.206	0.547	0.544	0.548	0.623	0.611	0.629	0.644	0.640	0.645	0.629	0.628	0.636	0.638	0.637	0.645
175.0	0.108	0.109	0.117	0.109	0.113	0.116	0.118	0.119	0.122	0.199	0.198	0.183	0.515	0.503	0.507	0.626	0.614	0.630	0.652	0.650	0.653	0.639	0.635	0.643	0.648	0.643	0.652
185.5	0.107	0.109	0.117	0.109	0.113	0.116	0.118	0.118	0.122	0.183	0.184	0.168	0.478	0.457	0.452	0.625	0.615	0.630	0.655	0.653	0.657	0.642	0.641	0.647	0.651	0.650	0.655
196.0	0.107	0.109	0.118	0.108	0.114	0.116	0.117	0.118	0.121	0.172	0.175	0.159	0.435	0.404	0.390	0.624	0.615	0.630	0.662	0.659	0.664	0.649	0.650	0.654	0.658	0.658	0.663

206.5	**0.107**	**0.109**	**0.118**	**0.108**	**0.112**	**0.115**	**0.118**	**0.118**	**0.120**	**0.164**	**0.166**	**0.151**	**0.387**	**0.351**	**0.338**	**0.620**	**0.615**	**0.626**	**0.667**	**0.663**	**0.669**	**0.657**	**0.657**	**0.662**	**0.666**	**0.666**	**0.670**
217.0	**0.107**	**0.110**	**0.118**	**0.108**	**0.111**	**0.116**	**0.118**	**0.118**	**0.120**	**0.156**	**0.159**	**0.146**	**0.345**	**0.306**	**0.297**	**0.616**	**0.613**	**0.622**	**0.671**	**0.667**	**0.676**	**0.660**	**0.659**	**0.667**	**0.669**	**0.668**	**0.676**
227.5	**0.107**	**0.109**	**0.119**	**0.108**	**0.113**	**0.116**	**0.118**	**0.119**	**0.120**	**0.150**	**0.152**	**0.142**	**0.310**	**0.278**	**0.271**	**0.610**	**0.610**	**0.616**	**0.676**	**0.672**	**0.682**	**0.668**	**0.669**	**0.675**	**0.677**	**0.677**	**0.683**
238.0	**0.107**	**0.109**	**0.118**	**0.108**	**0.112**	**0.116**	**0.119**	**0.118**	**0.120**	**0.145**	**0.147**	**0.140**	**0.287**	**0.264**	**0.261**	**0.603**	**0.604**	**0.607**	**0.682**	**0.678**	**0.688**	**0.674**	**0.672**	**0.682**	**0.683**	**0.681**	**0.691**
248.5	**0.107**	**0.109**	**0.118**	**0.108**	**0.112**	**0.116**	**0.118**	**0.118**	**0.120**	**0.140**	**0.142**	**0.136**	**0.275**	**0.260**	**0.260**	**0.590**	**0.595**	**0.594**	**0.687**	**0.681**	**0.693**	**0.681**	**0.681**	**0.688**	**0.689**	**0.690**	**0.697**
259.0	**0.107**	**0.109**	**0.120**	**0.108**	**0.112**	**0.116**	**0.118**	**0.118**	**0.120**	**0.137**	**0.138**	**0.136**	**0.271**	**0.260**	**0.259**	**0.573**	**0.582**	**0.578**	**0.690**	**0.687**	**0.697**	**0.687**	**0.687**	**0.696**	**0.696**	**0.696**	**0.705**
269.5	**0.107**	**0.109**	**0.120**	**0.108**	**0.111**	**0.116**	**0.118**	**0.117**	**0.120**	**0.134**	**0.136**	**0.135**	**0.270**	**0.261**	**0.254**	**0.549**	**0.562**	**0.554**	**0.695**	**0.690**	**0.702**	**0.697**	**0.697**	**0.703**	**0.706**	**0.705**	**0.711**

2.5x10^7 cfu/ml

time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5.5	**0.109**	**0.123**	**0.125**	**0.128**	**0.132**	**0.134**	**0.133**	**0.134**	**0.135**	**0.133**	**0.141**	**0.139**	**0.133**	**0.136**	**0.137**	**0.140**	**0.138**	**0.139**	**0.134**	**0.137**	**0.138**	**0.134**	**0.137**	**0.136**	**0.136**	**0.136**	**0.137**
11.0	**0.119**	**0.123**	**0.126**	**0.127**	**0.131**	**0.134**	**0.134**	**0.136**	**0.134**	**0.136**	**0.142**	**0.139**	**0.135**	**0.138**	**0.139**	**0.140**	**0.138**	**0.141**	**0.137**	**0.138**	**0.139**	**0.138**	**0.138**	**0.137**	**0.139**	**0.139**	**0.139**
16.5	**0.118**	**0.121**	**0.125**	**0.126**	**0.130**	**0.134**	**0.136**	**0.138**	**0.137**	**0.139**	**0.147**	**0.143**	**0.139**	**0.141**	**0.144**	**0.143**	**0.143**	**0.144**	**0.140**	**0.142**	**0.142**	**0.141**	**0.142**	**0.141**	**0.143**	**0.145**	**0.142**
22.0	**0.109**	**0.113**	**0.117**	**0.118**	**0.121**	**0.126**	**0.138**	**0.140**	**0.140**	**0.144**	**0.150**	**0.147**	**0.142**	**0.146**	**0.149**	**0.147**	**0.147**	**0.149**	**0.145**	**0.147**	**0.148**	**0.147**	**0.148**	**0.146**	**0.151**	**0.150**	**0.150**
27.5	**0.101**	**0.105**	**0.110**	**0.110**	**0.114**	**0.118**	**0.141**	**0.142**	**0.141**	**0.149**	**0.156**	**0.152**	**0.148**	**0.151**	**0.155**	**0.155**	**0.153**	**0.153**	**0.151**	**0.153**	**0.155**	**0.154**	**0.154**	**0.153**	**0.159**	**0.158**	**0.156**
33.0	**0.097**	**0.100**	**0.106**	**0.105**	**0.108**	**0.115**	**0.143**	**0.145**	**0.142**	**0.156**	**0.163**	**0.159**	**0.156**	**0.159**	**0.164**	**0.162**	**0.160**	**0.163**	**0.160**	**0.161**	**0.163**	**0.162**	**0.163**	**0.160**	**0.170**	**0.169**	**0.166**
38.5	**0.094**	**0.098**	**0.101**	**0.103**	**0.106**	**0.109**	**0.145**	**0.147**	**0.147**	**0.165**	**0.173**	**0.167**	**0.164**	**0.168**	**0.173**	**0.171**	**0.169**	**0.169**	**0.169**	**0.170**	**0.172**	**0.173**	**0.173**	**0.170**	**0.183**	**0.182**	**0.178**
44.0	**0.094**	**0.096**	**0.101**	**0.103**	**0.105**	**0.110**	**0.150**	**0.151**	**0.152**	**0.175**	**0.183**	**0.178**	**0.174**	**0.178**	**0.184**	**0.181**	**0.180**	**0.181**	**0.181**	**0.181**	**0.183**	**0.187**	**0.185**	**0.183**	**0.200**	**0.197**	**0.193**
49.5	**0.094**	**0.095**	**0.100**	**0.103**	**0.104**	**0.109**	**0.155**	**0.158**	**0.158**	**0.189**	**0.197**	**0.190**	**0.188**	**0.193**	**0.197**	**0.196**	**0.193**	**0.194**	**0.196**	**0.196**	**0.197**	**0.203**	**0.201**	**0.199**	**0.219**	**0.216**	**0.210**
55.0	**0.095**	**0.095**	**0.100**	**0.104**	**0.104**	**0.108**	**0.163**	**0.165**	**0.164**	**0.203**	**0.213**	**0.204**	**0.203**	**0.209**	**0.214**	**0.210**	**0.208**	**0.208**	**0.212**	**0.211**	**0.214**	**0.221**	**0.219**	**0.215**	**0.240**	**0.236**	**0.229**
66.0	**0.097**	**0.095**	**0.100**	**0.106**	**0.104**	**0.109**	**0.179**	**0.179**	**0.180**	**0.241**	**0.255**	**0.246**	**0.243**	**0.253**	**0.255**	**0.249**	**0.250**	**0.248**	**0.256**	**0.256**	**0.257**	**0.267**	**0.266**	**0.261**	**0.302**	**0.290**	**0.277**
71.5	**0.096**	**0.095**	**0.099**	**0.104**	**0.104**	**0.108**	**0.183**	**0.184**	**0.185**	**0.258**	**0.274**	**0.265**	**0.263**	**0.273**	**0.278**	**0.270**	**0.272**	**0.269**	**0.281**	**0.276**	**0.278**	**0.290**	**0.287**	**0.283**	**0.331**	**0.318**	**0.302**
77.0	**0.098**	**0.095**	**0.099**	**0.107**	**0.104**	**0.108**	**0.186**	**0.188**	**0.188**	**0.273**	**0.292**	**0.280**	**0.282**	**0.293**	**0.296**	**0.290**	**0.291**	**0.287**	**0.300**	**0.295**	**0.298**	**0.312**	**0.309**	**0.303**	**0.351**	**0.340**	**0.324**
92.5	**0.094**	**0.095**	**0.099**	**0.103**	**0.104**	**0.108**	**0.187**	**0.192**	**0.191**	**0.323**	**0.343**	**0.332**	**0.343**	**0.355**	**0.362**	**0.351**	**0.359**	**0.348**	**0.365**	**0.359**	**0.361**	**0.379**	**0.377**	**0.361**	**0.414**	**0.404**	**0.385**
99.0	**0.094**	**0.095**	**0.099**	**0.103**	**0.104**	**0.108**	**0.187**	**0.192**	**0.191**	**0.323**	**0.343**	**0.332**	**0.343**	**0.355**	**0.362**	**0.351**	**0.359**	**0.348**	**0.365**	**0.359**	**0.361**	**0.379**	**0.377**	**0.361**	**0.414**	**0.404**	**0.385**
105.5	**0.095**	**0.095**	**0.099**	**0.104**	**0.104**	**0.108**	**0.181**	**0.185**	**0.184**	**0.337**	**0.359**	**0.345**	**0.366**	**0.378**	**0.384**	**0.375**	**0.381**	**0.370**	**0.386**	**0.383**	**0.384**	**0.401**	**0.402**	**0.385**	**0.436**	**0.431**	**0.412**
112.0	**0.094**	**0.094**	**0.099**	**0.103**	**0.103**	**0.107**	**0.168**	**0.173**	**0.170**	**0.345**	**0.366**	**0.355**	**0.387**	**0.402**	**0.409**	**0.398**	**0.406**	**0.395**	**0.412**	**0.405**	**0.407**	**0.427**	**0.426**	**0.409**	**0.457**	**0.454**	**0.436**
118.5	**0.094**	**0.094**	**0.097**	**0.103**	**0.103**	**0.106**	**0.151**	**0.153**	**0.151**	**0.350**	**0.368**	**0.357**	**0.410**	**0.422**	**0.430**	**0.423**	**0.430**	**0.419**	**0.436**	**0.428**	**0.433**	**0.449**	**0.448**	**0.432**	**0.477**	**0.474**	**0.458**
125.0	**0.095**	**0.094**	**0.097**	**0.104**	**0.103**	**0.106**	**0.135**	**0.135**	**0.135**	**0.345**	**0.363**	**0.355**	**0.426**	**0.439**	**0.446**	**0.445**	**0.449**	**0.439**	**0.455**	**0.448**	**0.453**	**0.466**	**0.465**	**0.450**	**0.494**	**0.493**	**0.478**
131.5	**0.093**	**0.094**	**0.097**	**0.102**	**0.102**	**0.106**	**0.123**	**0.122**	**0.122**	**0.328**	**0.340**	**0.337**	**0.433**	**0.449**	**0.453**	**0.459**	**0.466**	**0.457**	**0.472**	**0.467**	**0.469**	**0.484**	**0.484**	**0.469**	**0.512**	**0.514**	**0.496**
138.0	**0.094**	**0.094**	**0.097**	**0.103**	**0.102**	**0.106**	**0.117**	**0.116**	**0.115**	**0.291**	**0.305**	**0.299**	**0.441**	**0.456**	**0.464**	**0.477**	**0.483**	**0.475**	**0.491**	**0.487**	**0.492**	**0.502**	**0.504**	**0.486**	**0.526**	**0.529**	**0.515**

144.5	0.093	0.093	0.096	0.102	0.102	0.105	0.112	0.112	0.112	0.243	0.255	0.247	0.442	0.457	0.465	0.492	0.501	0.490	0.508	0.505	0.510	0.518	0.520	0.504	0.539	0.543	0.528
151.0	0.093	0.093	0.097	0.102	0.102	0.106	0.111	0.110	0.110	0.194	0.204	0.199	0.434	0.449	0.457	0.503	0.512	0.501	0.523	0.518	0.523	0.531	0.532	0.520	0.545	0.552	0.539
157.5	0.094	0.093	0.097	0.103	0.102	0.106	0.110	0.109	0.108	0.160	0.167	0.165	0.418	0.430	0.438	0.512	0.519	0.508	0.533	0.530	0.535	0.539	0.543	0.530	0.552	0.559	0.547
164.0	0.092	0.093	0.096	0.101	0.101	0.105	0.108	0.108	0.107	0.141	0.146	0.146	0.390	0.403	0.411	0.514	0.520	0.511	0.539	0.536	0.540	0.546	0.549	0.537	0.559	0.565	0.552
180.0	0.092	0.093	0.096	0.101	0.102	0.105	0.106	0.107	0.106	0.118	0.123	0.122	0.260	0.265	0.269	0.509	0.518	0.496	0.555	0.554	0.554	0.565	0.567	0.556	0.574	0.582	0.564
185.5	0.092	0.093	0.095	0.101	0.101	0.104	0.106	0.106	0.105	0.117	0.121	0.119	0.236	0.242	0.245	0.497	0.501	0.484	0.552	0.554	0.552	0.565	0.565	0.553	0.568	0.577	0.564
191.0	0.092	0.093	0.096	0.101	0.101	0.105	0.106	0.105	0.104	0.115	0.120	0.119	0.220	0.226	0.229	0.493	0.497	0.479	0.553	0.555	0.553	0.562	0.566	0.556	0.572	0.582	0.566
196.5	0.093	0.092	0.095	0.102	0.101	0.104	0.105	0.104	0.104	0.115	0.118	0.118	0.210	0.215	0.218	0.488	0.491	0.472	0.553	0.557	0.555	0.567	0.570	0.559	0.577	0.585	0.569
202.0	0.091	0.092	0.095	0.100	0.101	0.104	0.105	0.105	0.104	0.114	0.118	0.117	0.202	0.206	0.210	0.485	0.486	0.465	0.552	0.560	0.556	0.571	0.576	0.564	0.580	0.589	0.574
207.5	0.092	0.092	0.095	0.101	0.101	0.104	0.105	0.104	0.104	0.114	0.117	0.117	0.196	0.201	0.204	0.476	0.478	0.456	0.553	0.562	0.556	0.574	0.578	0.567	0.584	0.592	0.578
213.0	0.092	0.092	0.095	0.101	0.101	0.104	0.104	0.104	0.103	0.113	0.117	0.116	0.192	0.196	0.201	0.467	0.466	0.441	0.554	0.564	0.556	0.579	0.578	0.570	0.588	0.597	0.583
218.5	0.092	0.092	0.094	0.101	0.101	0.103	0.103	0.104	0.103	0.113	0.116	0.115	0.190	0.192	0.197	0.454	0.453	0.427	0.554	0.567	0.557	0.582	0.583	0.574	0.593	0.600	0.586
224.0	0.092	0.092	0.096	0.100	0.101	0.105	0.104	0.103	0.103	0.114	0.116	0.116	0.187	0.191	0.196	0.440	0.438	0.412	0.554	0.568	0.557	0.587	0.586	0.578	0.596	0.604	0.589
229.5	0.092	0.093	0.096	0.101	0.101	0.104	0.103	0.103	0.104	0.113	0.116	0.115	0.186	0.189	0.194	0.427	0.424	0.397	0.553	0.568	0.555	0.588	0.590	0.580	0.600	0.607	0.592

10^7 cfu/ml																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
3.5	0.083	0.095	0.100	0.102	0.103	0.109	0.104	0.107	0.145	0.103	0.111	0.109	0.103	0.105	0.107	0.104	0.107	0.110	0.104	0.106	0.105	0.102	0.107	0.102	0.104	0.106	0.106
14.0	0.093	0.094	0.099	0.102	0.103	0.108	0.104	0.106	0.143	0.103	0.112	0.110	0.103	0.105	0.110	0.104	0.108	0.111	0.103	0.105	0.106	0.103	0.107	0.103	0.104	0.106	0.106
24.5	0.093	0.094	0.100	0.102	0.103	0.108	0.105	0.108	0.142	0.104	0.113	0.112	0.104	0.107	0.112	0.106	0.108	0.116	0.105	0.107	0.108	0.105	0.112	0.106	0.108	0.110	0.111
31.5	0.087	0.092	0.095	0.096	0.101	0.104	0.118	0.139	0.136	0.108	0.129	0.115	0.108	0.112	0.117	0.109	0.115	0.119	0.109	0.112	0.114	0.110	0.120	0.112	0.115	0.117	0.117
42.0	0.083	0.086	0.093	0.092	0.095	0.101	0.109	0.114	0.137	0.113	0.124	0.121	0.113	0.118	0.125	0.114	0.123	0.128	0.115	0.119	0.121	0.114	0.126	0.119	0.122	0.127	0.125
52.5	0.082	0.086	0.094	0.091	0.095	0.103	0.111	0.115	0.138	0.120	0.135	0.131	0.122	0.127	0.135	0.123	0.132	0.139	0.124	0.128	0.133	0.125	0.136	0.132	0.135	0.139	0.140
59.5	0.083	0.092	0.096	0.092	0.101	0.105	0.114	0.144	0.140	0.129	0.144	0.141	0.132	0.139	0.147	0.133	0.143	0.150	0.135	0.139	0.146	0.135	0.149	0.143	0.149	0.154	0.156
70.0	0.083	0.091	0.098	0.092	0.100	0.106	0.117	0.124	0.143	0.140	0.159	0.156	0.145	0.156	0.165	0.146	0.161	0.171	0.150	0.156	0.164	0.150	0.166	0.161	0.169	0.173	0.178
80.5	0.083	0.092	0.097	0.092	0.101	0.106	0.120	0.128	0.144	0.156	0.179	0.177	0.165	0.178	0.194	0.165	0.182	0.199	0.172	0.180	0.191	0.173	0.188	0.188	0.199	0.206	0.214
94.5	0.083	0.093	0.098	0.092	0.102	0.107	0.121	0.136	0.142	0.184	0.211	0.207	0.203	0.223	0.246	0.203	0.227	0.252	0.214	0.226	0.243	0.217	0.236	0.241	0.254	0.260	0.271
105.0	0.084	0.096	0.100	0.093	0.105	0.109	0.119	0.127	0.134	0.203	0.231	0.223	0.238	0.262	0.284	0.240	0.270	0.294	0.254	0.267	0.286	0.258	0.282	0.284	0.300	0.310	0.320
115.5	0.085	0.095	0.099	0.094	0.104	0.108	0.113	0.119	0.125	0.211	0.237	0.226	0.273	0.299	0.315	0.285	0.315	0.343	0.299	0.316	0.337	0.306	0.333	0.337	0.350	0.356	0.361
126.0	0.085	0.096	0.100	0.094	0.105	0.109	0.104	0.114	0.119	0.205	0.230	0.210	0.304	0.324	0.335	0.329	0.360	0.383	0.349	0.362	0.380	0.353	0.378	0.379	0.387	0.391	0.396
133.0	0.086	0.095	0.101	0.095	0.104	0.110	0.104	0.113	0.117	0.194	0.212	0.187	0.316	0.333	0.336	0.358	0.385	0.403	0.376	0.390	0.403	0.381	0.405	0.406	0.410	0.416	0.421

143.5	0.087	0.096	0.101	0.096	0.105	0.110	0.103	0.113	0.116	0.157	0.166	0.146	0.311	0.316	0.298	0.385	0.407	0.420	0.415	0.426	0.442	0.421	0.446	0.446	0.451	0.456	0.463
150.5	0.088	0.098	0.104	0.097	0.107	0.112	0.103	0.115	0.114	0.136	0.146	0.133	0.289	0.287	0.253	0.394	0.415	0.419	0.443	0.448	0.463	0.449	0.471	0.471	0.477	0.479	0.486
154.0	0.085	0.095	0.099	0.098	0.108	0.113	0.102	0.114	0.113	0.128	0.138	0.130	0.271	0.263	0.228	0.394	0.415	0.412	0.455	0.460	0.472	0.465	0.488	0.487	0.490	0.494	0.498
164.5	0.085	0.096	0.100	0.099	0.110	0.115	0.102	0.115	0.112	0.118	0.128	0.124	0.206	0.198	0.170	0.389	0.395	0.347	0.482	0.476	0.479	0.501	0.520	0.516	0.516	0.519	0.521
175.0	0.086	0.095	0.101	0.102	0.114	0.118	0.104	0.119	0.112	0.114	0.123	0.120	0.165	0.165	0.146	0.327	0.316	0.255	0.493	0.466	0.468	0.527	0.544	0.531	0.539	0.537	0.534
185.5	0.087	0.096	0.101	0.104	0.115	0.122	0.105	0.120	0.112	0.111	0.121	0.118	0.149	0.149	0.138	0.244	0.238	0.200	0.484	0.430	0.417	0.538	0.549	0.528	0.548	0.547	0.543
196.0	0.088	0.098	0.104	0.107	0.119	0.126	0.106	0.121	0.114	0.110	0.119	0.118	0.138	0.142	0.133	0.195	0.196	0.177	0.440	0.348	0.323	0.529	0.540	0.513	0.555	0.553	0.549
206.5	0.085	0.095	0.099	0.112	0.124	0.131	0.107	0.124	0.115	0.110	0.119	0.117	0.133	0.141	0.130	0.171	0.177	0.165	0.359	0.272	0.267	0.499	0.515	0.490	0.562	0.560	0.554
217.0	0.085	0.096	0.100	0.116	0.128	0.135	0.110	0.129	0.116	0.110	0.120	0.119	0.131	0.140	0.130	0.159	0.166	0.156	0.300	0.238	0.243	0.465	0.488	0.460	0.570	0.568	0.560
227.5	0.086	0.095	0.101	0.122	0.132	0.139	0.112	0.131	0.117	0.111	0.121	0.121	0.132	0.143	0.130	0.152	0.159	0.154	0.266	0.229	0.236	0.433	0.457	0.428	0.576	0.573	0.567
238.0	0.087	0.096	0.101	0.127	0.138	0.143	0.114	0.135	0.120	0.113	0.123	0.124	0.134	0.146	0.132	0.150	0.157	0.153	0.251	0.225	0.235	0.393	0.421	0.396	0.588	0.584	0.575
248.5	0.088	0.098	0.104	0.133	0.144	0.149	0.118	0.140	0.121	0.116	0.127	0.126	0.137	0.149	0.135	0.150	0.157	0.154	0.244	0.226	0.238	0.356	0.387	0.366	0.597	0.592	0.582
259.0	0.085	0.095	0.099	0.139	0.149	0.151	0.119	0.144	0.122	0.118	0.131	0.131	0.139	0.153	0.137	0.151	0.158	0.154	0.245	0.227	0.241	0.326	0.350	0.333	0.606	0.602	0.590
269.5	0.085	0.096	0.100	0.144	0.154	0.157	0.119	0.146	0.125	0.122	0.135	0.136	0.143	0.158	0.143	0.155	0.161	0.158	0.246	0.229	0.243	0.293	0.312	0.301	0.613	0.608	0.595

5x10^6 cfu/ml																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
3.5	0.077	0.090	0.087	0.096	0.099	0.096	0.098	0.106	0.098	0.096	0.101	0.100	0.100	0.105	0.100	0.111	0.111	0.100	0.096	0.111	0.102	0.106	0.119	0.097	0.125	0.122	0.104
14.0	0.088	0.091	0.087	0.096	0.099	0.096	0.098	0.106	0.099	0.096	0.102	0.101	0.102	0.106	0.100	0.112	0.112	0.101	0.097	0.114	0.104	0.104	0.121	0.097	0.118	0.119	0.107
24.5	0.089	0.093	0.088	0.098	0.102	0.097	0.099	0.109	0.101	0.098	0.103	0.103	0.103	0.108	0.102	0.113	0.113	0.103	0.098	0.115	0.108	0.107	0.122	0.099	0.125	0.124	0.110
31.5	0.090	0.096	0.089	0.099	0.105	0.098	0.102	0.116	0.105	0.101	0.110	0.106	0.109	0.116	0.106	0.122	0.121	0.110	0.102	0.122	0.112	0.119	0.129	0.103	0.133	0.135	0.112
42.0	0.089	0.096	0.087	0.097	0.104	0.096	0.103	0.118	0.107	0.104	0.115	0.110	0.113	0.122	0.111	0.130	0.127	0.114	0.105	0.129	0.115	0.119	0.134	0.109	0.129	0.134	0.118
52.5	0.085	0.093	0.085	0.094	0.101	0.094	0.103	0.118	0.106	0.109	0.120	0.115	0.119	0.126	0.118	0.133	0.134	0.120	0.111	0.135	0.123	0.129	0.140	0.115	0.140	0.143	0.127
59.5	0.085	0.093	0.086	0.093	0.101	0.094	0.105	0.119	0.106	0.116	0.125	0.122	0.131	0.133	0.125	0.143	0.138	0.128	0.119	0.137	0.131	0.134	0.147	0.125	0.151	0.147	0.136
70.0	0.084	0.092	0.086	0.093	0.101	0.095	0.104	0.117	0.107	0.121	0.132	0.128	0.135	0.143	0.137	0.143	0.146	0.140	0.127	0.149	0.141	0.142	0.159	0.136	0.150	0.157	0.151
80.5	0.083	0.093	0.086	0.092	0.102	0.095	0.104	0.119	0.105	0.127	0.138	0.134	0.151	0.157	0.151	0.160	0.165	0.156	0.141	0.163	0.160	0.159	0.171	0.157	0.174	0.176	0.172
94.5	0.082	0.092	0.086	0.091	0.101	0.095	0.100	0.110	0.101	0.130	0.141	0.135	0.171	0.174	0.172	0.190	0.187	0.188	0.169	0.193	0.194	0.190	0.205	0.192	0.205	0.211	0.214
105.0	0.083	0.093	0.087	0.092	0.102	0.096	0.097	0.105	0.099	0.128	0.136	0.136	0.179	0.184	0.184	0.210	0.214	0.219	0.196	0.219	0.230	0.217	0.233	0.230	0.242	0.247	0.257
115.5	0.083	0.092	0.087	0.092	0.101	0.095	0.096	0.103	0.097	0.128	0.133	0.131	0.187	0.191	0.191	0.234	0.239	0.242	0.231	0.253	0.267	0.254	0.275	0.267	0.278	0.286	0.299
126.0	0.084	0.094	0.087	0.092	0.103	0.096	0.095	0.104	0.098	0.123	0.126	0.123	0.185	0.185	0.186	0.249	0.253	0.253	0.264	0.282	0.297	0.297	0.314	0.316	0.325	0.335	0.350
129.5	0.083	0.093	0.087	0.092	0.102	0.096	0.095	0.104	0.097	0.119	0.123	0.119	0.181	0.179	0.178	0.247	0.257	0.262	0.281	0.302	0.310	0.329	0.346	0.343	0.358	0.366	0.374

143.5	0.083	0.092	0.088	0.092	0.101	0.097	0.094	0.104	0.096	0.109	0.115	0.113	0.163	0.160	0.148	0.247	0.257	0.262	0.295	0.311	0.321	0.360	0.377	0.372	0.388	0.397	0.410
150.5	0.084	0.093	0.088	0.092	0.102	0.096	0.095	0.103	0.095	0.105	0.112	0.110	0.147	0.141	0.128	0.247	0.253	0.247	0.305	0.317	0.322	0.376	0.391	0.384	0.412	0.424	0.439
154.0	0.083	0.093	0.088	0.092	0.102	0.097	0.094	0.104	0.096	0.104	0.111	0.110	0.137	0.134	0.121	0.239	0.246	0.236	0.305	0.314	0.317	0.379	0.397	0.389	0.422	0.435	0.452
164.5	0.083	0.092	0.088	0.092	0.101	0.096	0.093	0.103	0.096	0.100	0.106	0.106	0.117	0.116	0.111	0.204	0.212	0.180	0.292	0.296	0.277	0.382	0.406	0.400	0.463	0.474	0.491
175.0	0.084	0.094	0.089	0.093	0.103	0.098	0.094	0.103	0.095	0.097	0.105	0.104	0.108	0.110	0.109	0.169	0.169	0.132	0.257	0.259	0.228	0.381	0.397	0.372	0.498	0.508	0.522
185.5	0.084	0.094	0.089	0.093	0.103	0.098	0.094	0.104	0.096	0.095	0.103	0.103	0.105	0.108	0.108	0.135	0.131	0.115	0.213	0.216	0.189	0.329	0.335	0.285	0.526	0.535	0.549
196.0	0.084	0.093	0.089	0.093	0.102	0.098	0.093	0.104	0.095	0.094	0.102	0.102	0.103	0.109	0.109	0.122	0.121	0.112	0.177	0.183	0.165	0.259	0.269	0.216	0.547	0.555	0.559
206.5	0.085	0.093	0.090	0.093	0.102	0.099	0.094	0.104	0.096	0.093	0.101	0.102	0.102	0.107	0.107	0.118	0.117	0.112	0.149	0.158	0.145	0.217	0.227	0.184	0.559	0.565	0.569
217.0	0.086	0.095	0.089	0.094	0.104	0.098	0.094	0.104	0.095	0.093	0.100	0.101	0.102	0.105	0.106	0.115	0.113	0.112	0.129	0.141	0.134	0.187	0.197	0.161	0.570	0.574	0.580
227.5	0.084	0.094	0.089	0.092	0.103	0.098	0.093	0.104	0.096	0.092	0.099	0.101	0.099	0.105	0.105	0.112	0.114	0.112	0.121	0.136	0.130	0.158	0.169	0.144	0.578	0.582	0.586
238.0	0.085	0.094	0.092	0.094	0.103	0.101	0.093	0.104	0.096	0.093	0.099	0.102	0.099	0.105	0.106	0.113	0.115	0.112	0.118	0.133	0.127	0.143	0.156	0.137	0.586	0.590	0.594
248.5	0.085	0.093	0.090	0.093	0.102	0.098	0.094	0.106	0.096	0.092	0.100	0.102	0.099	0.106	0.107	0.112	0.116	0.114	0.117	0.130	0.122	0.136	0.150	0.132	0.595	0.597	0.601
259.0	0.085	0.095	0.090	0.093	0.104	0.099	0.093	0.105	0.096	0.093	0.101	0.101	0.100	0.105	0.106	0.112	0.117	0.112	0.113	0.126	0.119	0.133	0.146	0.131	0.602	0.609	0.612
269.5	0.085	0.095	0.091	0.094	0.104	0.100	0.093	0.105	0.096	0.092	0.099	0.101	0.100	0.107	0.107	0.111	0.115	0.112	0.112	0.125	0.119	0.131	0.143	0.128	0.610	0.615	0.618

10^6 cfu/ml																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
70.0	0.071	0.082	0.089	0.090	0.091	0.098	0.093	0.091	0.095	0.114	0.124	0.102	0.101	0.114	0.104	0.101	0.100	0.107	0.105	0.102	0.117	0.101	0.108	0.105	0.105	0.114	0.131
120.5	0.082	0.083	0.090	0.091	0.092	0.099	0.102	0.102	0.095	0.121	0.129	0.114	0.137	0.146	0.135	0.144	0.139	0.147	0.146	0.144	0.160	0.139	0.145	0.150	0.147	0.150	0.173
131.0	0.082	0.083	0.089	0.091	0.092	0.098	0.091	0.091	0.094	0.108	0.110	0.111	0.141	0.145	0.143	0.152	0.145	0.158	0.152	0.156	0.174	0.158	0.158	0.170	0.166	0.169	0.192
141.5	0.082	0.083	0.088	0.091	0.091	0.097	0.091	0.090	0.095	0.104	0.105	0.107	0.144	0.150	0.144	0.168	0.162	0.176	0.173	0.178	0.199	0.182	0.181	0.198	0.194	0.194	0.223
155.5	0.083	0.083	0.088	0.092	0.092	0.097	0.091	0.091	0.095	0.096	0.097	0.099	0.143	0.146	0.140	0.185	0.177	0.186	0.209	0.213	0.238	0.226	0.223	0.247	0.247	0.243	0.271
166.0	0.082	0.083	0.091	0.091	0.092	0.100	0.090	0.091	0.096	0.092	0.093	0.096	0.128	0.128	0.123	0.189	0.181	0.191	0.240	0.238	0.259	0.259	0.257	0.287	0.289	0.284	0.315
176.5	0.082	0.083	0.091	0.091	0.092	0.100	0.090	0.090	0.097	0.091	0.092	0.095	0.110	0.109	0.107	0.183	0.176	0.183	0.260	0.249	0.268	0.288	0.290	0.323	0.335	0.326	0.360
187.0	0.082	0.083	0.091	0.091	0.092	0.100	0.091	0.090	0.095	0.091	0.092	0.094	0.099	0.100	0.100	0.164	0.158	0.158	0.269	0.255	0.271	0.304	0.312	0.348	0.370	0.361	0.394
194.0	0.082	0.083	0.091	0.091	0.092	0.100	0.091	0.091	0.097	0.091	0.092	0.094	0.096	0.097	0.098	0.147	0.142	0.137	0.271	0.253	0.261	0.307	0.319	0.357	0.393	0.380	0.419
204.5	0.082	0.083	0.091	0.091	0.092	0.100	0.090	0.090	0.094	0.091	0.092	0.094	0.094	0.097	0.097	0.118	0.116	0.113	0.257	0.227	0.222	0.292	0.323	0.356	0.432	0.420	0.454
211.5	0.082	0.083	0.093	0.091	0.092	0.101	0.090	0.090	0.095	0.090	0.092	0.093	0.094	0.095	0.097	0.106	0.104	0.108	0.233	0.204	0.197	0.271	0.319	0.335	0.460	0.449	0.485
215.0	0.083	0.083	0.093	0.091	0.092	0.102	0.090	0.091	0.096	0.090	0.091	0.093	0.094	0.096	0.097	0.102	0.101	0.108	0.219	0.191	0.187	0.256	0.311	0.311	0.473	0.461	0.495

225.5	0.083	0.083	0.092	0.091	0.092	0.101	0.091	0.090	0.097	0.091	0.091	0.093	0.094	0.096	0.096	0.098	0.097	0.108	0.191	0.163	0.161	0.218	0.270	0.250	0.509	0.498	0.534
236.0	0.082	0.083	0.095	0.091	0.092	0.104	0.091	0.091	0.096	0.091	0.091	0.093	0.094	0.096	0.096	0.097	0.097	0.104	0.172	0.141	0.138	0.193	0.235	0.216	0.538	0.524	0.558
246.5	0.082	0.083	0.095	0.091	0.092	0.103	0.091	0.091	0.094	0.090	0.092	0.093	0.093	0.096	0.097	0.097	0.098	0.103	0.154	0.125	0.130	0.172	0.211	0.192	0.557	0.540	0.575
257.0	0.083	0.083	0.095	0.092	0.092	0.104	0.090	0.090	0.094	0.090	0.092	0.093	0.093	0.096	0.097	0.097	0.098	0.102	0.138	0.119	0.127	0.155	0.189	0.172	0.569	0.552	0.586
267.5	0.082	0.083	0.096	0.091	0.092	0.104	0.091	0.091	0.096	0.091	0.091	0.093	0.094	0.096	0.096	0.098	0.098	0.105	0.125	0.115	0.120	0.139	0.168	0.155	0.575	0.562	0.597
278.0	0.083	0.083	0.095	0.092	0.092	0.104	0.091	0.091	0.097	0.090	0.092	0.093	0.094	0.096	0.097	0.098	0.098	0.103	0.120	0.115	0.121	0.131	0.154	0.147	0.583	0.568	0.605
288.5	0.083	0.083	0.098	0.092	0.092	0.107	0.091	0.091	0.096	0.092	0.091	0.093	0.094	0.096	0.096	0.098	0.099	0.106	0.119	0.113	0.119	0.126	0.146	0.140	0.594	0.574	0.614

5x10^5 cfu/ml																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
100.0	0.099	0.100	0.102	0.099	0.100	0.102	0.099	0.100	0.102	0.114	0.124	0.102	0.101	0.114	0.104	0.101	0.100	0.107	0.105	0.102	0.117	0.101	0.108	0.105	0.105	0.114	0.131
130.0																											
156.0	0.099	0.104	0.102	0.099	0.104	0.102	0.099	0.104	0.102	0.099	0.104	0.102	0.113	0.113	0.113	0.137	0.117	0.119	0.134	0.138	0.142	0.126	0.124	0.122	0.134	0.131	0.134
159.0	0.099	0.101	0.100	0.099	0.101	0.100	0.099	0.101	0.100	0.099	0.101	0.100	0.115	0.113	0.111	0.134	0.129	0.122	0.134	0.138	0.141	0.125	0.124	0.124	0.133	0.132	0.134
162.0	0.099	0.102	0.102	0.099	0.102	0.102	0.099	0.102	0.102	0.099	0.102	0.102	0.113	0.111	0.111	0.135	0.139	0.142	0.134	0.138	0.141	0.123	0.123	0.124	0.134	0.134	0.135
165.0	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.111	0.110	0.111	0.136	0.139	0.142	0.134	0.138	0.132	0.118	0.121	0.116	0.136	0.135	0.138
168.0	0.097	0.101	0.101	0.097	0.101	0.101	0.097	0.101	0.101	0.097	0.101	0.101	0.110	0.110	0.108	0.134	0.138	0.142	0.142	0.143	0.135	0.122	0.126	0.121	0.138	0.139	0.141
171.0	0.099	0.100	0.102	0.099	0.100	0.102	0.099	0.100	0.102	0.099	0.100	0.102	0.111	0.109	0.107	0.134	0.138	0.141	0.143	0.155	0.167	0.126	0.125	0.126	0.141	0.141	0.143
174.0	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.110	0.109	0.109	0.134	0.138	0.141	0.142	0.143	0.165	0.136	0.135	0.133	0.145	0.145	0.149
177.0	0.099	0.104	0.102	0.099	0.104	0.102	0.099	0.104	0.102	0.099	0.104	0.102	0.113	0.113	0.113	0.134	0.138	0.132	0.146	0.157	0.174	0.136	0.139	0.139	0.149	0.149	0.151
180.0	0.099	0.101	0.100	0.099	0.101	0.100	0.099	0.101	0.100	0.099	0.101	0.100	0.115	0.113	0.111	0.134	0.149	0.122	0.148	0.159	0.177	0.136	0.131	0.131	0.153	0.154	0.156
183.0	0.099	0.102	0.102	0.099	0.102	0.102	0.099	0.102	0.102	0.099	0.102	0.102	0.113	0.111	0.111	0.135	0.139	0.142	0.150	0.163	0.179	0.136	0.137	0.137	0.164	0.164	0.165
186.0	0.099	0.100	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.102	0.111	0.110	0.111	0.136	0.139	0.142	0.153	0.165	0.180	0.139	0.144	0.148	0.171	0.168	0.177
189.0	0.099	0.101	0.102	0.097	0.101	0.101	0.097	0.101	0.101	0.097	0.101	0.101	0.110	0.110	0.108	0.134	0.138	0.142	0.158	0.170	0.187	0.153	0.157	0.158	0.174	0.174	0.184
192.0	0.099	0.104	0.102	0.099	0.101	0.102	0.099	0.100	0.102	0.099	0.100	0.102	0.111	0.109	0.107	0.134	0.138	0.141	0.161	0.175	0.190	0.155	0.162	0.170	0.184	0.182	0.194
195.0	0.099	0.101	0.100	0.099	0.104	0.102	0.099	0.101	0.102	0.098	0.099	0.102	0.110	0.109	0.109	0.134	0.138	0.140	0.165	0.180	0.196	0.163	0.173	0.181	0.190	0.190	0.200
198.0	0.099	0.102	0.102	0.099	0.101	0.100	0.099	0.104	0.102	0.099	0.100	0.102	0.108	0.108	0.109	0.135	0.139	0.141	0.170	0.185	0.198	0.182	0.182	0.198	0.200	0.199	0.213
201.0	0.099	0.101	0.102	0.099	0.102	0.102	0.099	0.101	0.100	0.097	0.098	0.102	0.106	0.108	0.106	0.133	0.139	0.142				0.197	0.206	0.213	0.214	0.213	0.225
204.0	0.097	0.101	0.101	0.099	0.101	0.102	0.099	0.102	0.102	0.101	0.101	0.102	0.110	0.108	0.106	0.134	0.137	0.140	0.183	0.202	0.211	0.205	0.217	0.227	0.223	0.224	0.239
207.0	0.099	0.100	0.102	0.097	0.101	0.101	0.099	0.101	0.102	0.098	0.098	0.102	0.107	0.107	0.107	0.134	0.138	0.140	0.186	0.204	0.213	0.214	0.231	0.244	0.236	0.234	0.251

210.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.097**	**0.101**	**0.101**	**0.097**	**0.097**	**0.101**	**0.106**	**0.106**	**0.106**	**0.134**	**0.137**	**0.140**	**0.188**	**0.207**	**0.215**	**0.228**	**0.246**	**0.261**			
213.0	**0.099**	**0.104**	**0.102**	**0.099**	**0.101**	**0.100**	**0.099**	**0.100**	**0.102**	**0.096**	**0.100**	**0.102**	**0.105**	**0.106**	**0.106**	**0.134**	**0.137**	**0.141**	**0.189**	**0.209**	**0.217**	**0.243**	**0.260**	**0.277**	**0.272**	**0.273**	**0.289**
216.0	**0.099**	**0.100**	**0.102**	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.097**	**0.102**	**0.106**	**0.106**	**0.106**	**0.133**	**0.137**	**0.139**	**0.191**	**0.211**	**0.219**	**0.256**	**0.274**	**0.271**	**0.287**	**0.289**	**0.305**
219.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.098**	**0.098**	**0.103**															
223.0	**0.099**	**0.104**	**0.102**	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.100**	**0.105**	**0.097**	**0.098**	**0.107**	**0.105**	**0.098**	**0.125**	**0.128**	**0.134**	**0.196**	**0.195**	**0.202**	**0.282**	**0.285**	**0.266**	**0.323**	**0.314**	**0.322**
226.0	**0.099**	**0.101**	**0.100**	**0.099**	**0.100**	**0.102**	**0.099**	**0.100**	**0.102**	**0.107**	**0.097**	**0.096**	**0.107**	**0.103**	**0.098**	**0.123**	**0.124**	**0.130**	**0.182**	**0.182**	**0.198**	**0.290**	**0.295**	**0.255**	**0.334**	**0.326**	**0.334**
229.0	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.105**	**0.096**	**0.097**	**0.106**	**0.105**	**0.097**	**0.120**	**0.122**	**0.129**	**0.169**	**0.171**	**0.188**	**0.290**	**0.297**	**0.247**	**0.342**	**0.339**	**0.345**
232.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.099**	**0.104**	**0.102**	**0.104**	**0.097**	**0.096**	**0.105**	**0.106**	**0.097**	**0.116**	**0.125**	**0.125**	**0.158**	**0.162**	**0.175**	**0.286**	**0.294**	**0.235**	**0.352**	**0.347**	**0.355**
235.0	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.100**	**0.099**	**0.101**	**0.100**	**0.103**	**0.098**	**0.097**	**0.104**	**0.106**	**0.096**	**0.114**	**0.121**	**0.123**	**0.147**	**0.154**	**0.164**	**0.281**	**0.295**	**0.219**	**0.365**	**0.360**	**0.367**
238.0	**0.099**	**0.100**	**0.102**	**0.099**	**0.102**	**0.102**	**0.099**	**0.102**	**0.102**	**0.106**	**0.098**	**0.098**	**0.105**	**0.106**	**0.096**	**0.111**	**0.118**	**0.122**	**0.139**	**0.145**	**0.154**	**0.273**	**0.290**	**0.202**	**0.375**	**0.367**	**0.378**
241.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.105**	**0.099**	**0.097**	**0.105**	**0.105**	**0.098**	**0.110**	**0.112**	**0.121**	**0.133**	**0.138**	**0.148**	**0.261**	**0.278**	**0.188**	**0.386**	**0.381**	**0.386**
244.0	**0.099**	**0.100**	**0.102**	**0.097**	**0.101**	**0.101**	**0.097**	**0.101**	**0.101**	**0.106**	**0.102**	**0.097**	**0.104**	**0.107**	**0.097**	**0.107**	**0.117**	**0.119**	**0.128**	**0.134**	**0.143**	**0.243**	**0.267**	**0.176**	**0.397**	**0.390**	**0.398**
247.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.100**	**0.099**	**0.100**	**0.102**	**0.110**	**0.099**	**0.097**	**0.104**	**0.105**	**0.096**	**0.105**	**0.112**	**0.118**	**0.124**	**0.130**	**0.137**	**0.223**	**0.245**	**0.167**	**0.407**	**0.400**	**0.406**
250.0	**0.099**	**0.104**	**0.102**	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.102**	**0.106**	**0.099**	**0.096**	**0.104**	**0.104**	**0.096**	**0.105**	**0.109**	**0.115**	**0.121**	**0.126**	**0.134**	**0.207**	**0.226**	**0.163**	**0.417**	**0.410**	**0.419**
253.0	**0.099**	**0.101**	**0.100**	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.110**	**0.099**	**0.097**	**0.104**	**0.104**	**0.096**	**0.103**	**0.107**	**0.116**	**0.119**	**0.122**	**0.131**	**0.195**	**0.211**	**0.157**	**0.428**	**0.421**	**0.430**
256.0	**0.099**	**0.102**	**0.102**	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.100**	**0.104**	**0.100**	**0.097**	**0.103**	**0.105**	**0.096**	**0.103**	**0.110**	**0.116**	**0.117**	**0.120**	**0.130**	**0.188**	**0.203**	**0.153**	**0.437**	**0.428**	**0.437**
259.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.100**	**0.102**	**0.099**	**0.102**	**0.102**	**0.107**	**0.100**	**0.099**	**0.103**	**0.106**	**0.097**	**0.102**	**0.110**	**0.116**	**0.115**	**0.118**	**0.128**	**0.184**	**0.198**	**0.150**	**0.448**	**0.439**	**0.448**
262.0	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.102**	**0.099**	**0.100**	**0.102**	**0.105**	**0.100**	**0.097**	**0.104**	**0.105**	**0.096**	**0.102**	**0.107**	**0.117**	**0.113**	**0.116**	**0.125**	**0.181**	**0.194**	**0.145**	**0.457**	**0.449**	**0.457**
265.0	**0.099**	**0.100**	**0.102**	**0.099**	**0.104**	**0.102**	**0.099**	**0.101**	**0.102**	**0.107**	**0.099**	**0.097**	**0.104**	**0.105**	**0.097**	**0.102**	**0.110**	**0.116**	**0.112**	**0.115**	**0.124**	**0.179**	**0.192**	**0.143**	**0.464**	**0.456**	**0.464**
268.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.100**	**0.099**	**0.104**	**0.102**	**0.113**	**0.100**	**0.097**	**0.103**	**0.107**	**0.096**	**0.102**	**0.110**	**0.117**	**0.111**	**0.113**	**0.123**	**0.176**	**0.191**	**0.141**	**0.473**	**0.464**	**0.473**
271.0	**0.099**	**0.104**	**0.102**	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.100**	**0.109**	**0.101**	**0.099**	**0.103**	**0.108**	**0.097**	**0.102**	**0.111**	**0.117**	**0.110**	**0.112**	**0.123**	**0.174**	**0.190**	**0.138**	**0.482**	**0.472**	**0.480**
274.0	**0.099**	**0.101**	**0.100**	**0.099**	**0.101**	**0.102**	**0.099**	**0.102**	**0.102**	**0.110**	**0.102**	**0.097**	**0.104**	**0.105**	**0.096**	**0.102**	**0.107**	**0.116**	**0.108**	**0.110**	**0.121**	**0.172**	**0.187**	**0.136**	**0.486**	**0.478**	**0.486**
277.0	**0.099**	**0.102**	**0.102**	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.102**	**0.110**	**0.101**	**0.098**	**0.104**	**0.106**	**0.098**	**0.102**	**0.110**	**0.118**	**0.108**	**0.111**	**0.120**	**0.170**	**0.187**	**0.135**	**0.494**	**0.486**	**0.494**
280.0	**0.099**	**0.100**	**0.102**	**0.099**	**0.102**	**0.102**	**0.097**	**0.101**	**0.101**	**0.112**	**0.106**	**0.098**	**0.103**	**0.106**	**0.098**	**0.102**	**0.110**	**0.117**	**0.107**	**0.110**	**0.120**	**0.168**	**0.185**	**0.133**	**0.500**	**0.489**	**0.499**
283.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.100**	**0.102**	**0.114**	**0.106**	**0.100**	**0.103**	**0.107**	**0.099**	**0.104**	**0.111**	**0.116**	**0.109**	**0.114**	**0.122**	**0.168**	**0.187**	**0.134**	**0.505**	**0.493**	**0.500**
286.0	**0.099**	**0.104**	**0.102**	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.102**	**0.113**	**0.104**	**0.101**	**0.103**	**0.104**	**0.099**	**0.104**	**0.108**	**0.115**	**0.108**	**0.111**	**0.122**	**0.167**	**0.184**	**0.132**	**0.507**	**0.498**	**0.506**
289.0	**0.099**	**0.101**	**0.100**	**0.099**	**0.100**	**0.102**	**0.099**	**0.104**	**0.102**	**0.112**	**0.104**	**0.101**	**0.104**	**0.105**	**0.099**	**0.104**	**0.110**	**0.118**	**0.108**	**0.112**	**0.122**	**0.165**	**0.184**	**0.132**	**0.512**	**0.503**	**0.511**
292.0	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.100**	**0.111**	**0.105**	**0.100**	**0.103**	**0.107**	**0.099**	**0.104**	**0.112**	**0.118**	**0.108**	**0.114**	**0.121**	**0.164**	**0.183**	**0.130**	**0.516**	**0.509**	**0.513**
295.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.099**	**0.102**	**0.102**	**0.111**	**0.103**	**0.100**	**0.103**	**0.105**	**0.099**	**0.104**	**0.109**	**0.117**	**0.107**	**0.111**	**0.119**	**0.163**	**0.181**	**0.128**	**0.521**	**0.512**	**0.519**
298.0	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.100**	**0.099**	**0.100**	**0.102**	**0.110**	**0.103**	**0.101**	**0.104**	**0.105**	**0.101**	**0.104**	**0.109**	**0.117**	**0.107**	**0.110**	**0.119**	**0.161**	**0.178**	**0.127**	**0.525**	**0.515**	**0.522**
301.0	**0.099**	**0.100**	**0.102**	**0.099**	**0.102**	**0.102**	**0.099**	**0.101**	**0.102**	**0.111**	**0.101**	**0.101**	**0.104**	**0.106**	**0.099**	**0.104**	**0.111**	**0.117**	**0.107**	**0.111**	**0.118**	**0.159**	**0.177**	**0.125**	**0.530**	**0.517**	**0.526**
304.0	**0.099**	**0.101**	**0.102**	**0.099**	**0.101**	**0.102**	**0.099**	**0.104**	**0.102**	**0.111**	**0.102**	**0.101**	**0.103**	**0.106**	**0.100**	**0.105**	**0.109**	**0.117**	**0.108**	**0.109**	**0.118**	**0.158**	**0.175**	**0.124**	**0.532**	**0.520**	**0.529**
307.0	**0.099**	**0.104**	**0.102**	**0.097**	**0.101**	**0.101**	**0.099**	**0.101**	**0.100**	**0.111**	**0.102**	**0.100**	**0.104**	**0.105**	**0.099**	**0.104**	**0.109**	**0.116**	**0.107**	**0.110**	**0.117**	**0.157**	**0.174**	**0.123**	**0.535**	**0.522**	**0.531**
310.0	**0.099**	**0.101**	**0.100**	**0.099**	**0.104**	**0.102**	**0.099**	**0.102**	**0.102**	**0.111**	**0.099**	**0.100**	**0.103**	**0.104**	**0.100**	**0.104**	**0.108**	**0.115**	**0.107**	**0.108**	**0.118**	**0.155**	**0.172**	**0.124**	**0.535**	**0.524**	**0.534**

313.0	0.099	0.100	0.102	0.099	0.101	0.100	0.099	0.101	0.102	0.112	0.102	0.101	0.103	0.107	0.100	0.104	0.109	0.116	0.107	0.110	0.117	0.154	0.171	0.123	0.541	0.530	0.539
316.0	0.099	0.101	0.102	0.099	0.102	0.102	0.097	0.101	0.101	0.116	0.103	0.102	0.104	0.106	0.100	0.104	0.110	0.116	0.108	0.110	0.117	0.152	0.171	0.121	0.543	0.531	0.540
319.0	0.099	0.104	0.102	0.099	0.101	0.102	0.099	0.100	0.102	0.114	0.101	0.101	0.104	0.104	0.098	0.104	0.109	0.114	0.107	0.109	0.116	0.152	0.170	0.121	0.546	0.535	0.542
322.0	0.099	0.101	0.100	0.097	0.101	0.101	0.099	0.101	0.102	0.114	0.103	0.103	0.103	0.105	0.100	0.104	0.110	0.117	0.107	0.110	0.118	0.150	0.167	0.121	0.547	0.533	0.545
325.0	0.099	0.102	0.102	0.099	0.100	0.102	0.099	0.104	0.102	0.114	0.102	0.103	0.104	0.105	0.100	0.104	0.109	0.116	0.108	0.110	0.117	0.149	0.166	0.121	0.553	0.537	0.548
328.0	0.099	0.101	0.102	0.099	0.101	0.102	0.099	0.101	0.100	0.112	0.102	0.102	0.103	0.106	0.100	0.104	0.110	0.118	0.108	0.111	0.117	0.148	0.167	0.120	0.553	0.542	0.550
331.0	0.097	0.101	0.101	0.099	0.104	0.102	0.099	0.100	0.102	0.111	0.101	0.101	0.103	0.105	0.099	0.104	0.110	0.116	0.108	0.111	0.116	0.148	0.166	0.120	0.557	0.545	0.553
334.0	0.099	0.100	0.102	0.099	0.101	0.100	0.099	0.101	0.102	0.112	0.102	0.101	0.103	0.106	0.100	0.104	0.111	0.117	0.107	0.111	0.116	0.147	0.165	0.119	0.560	0.549	0.556
337.0	0.099	0.101	0.102	0.099	0.100	0.102	0.099	0.104	0.102	0.112	0.102	0.104	0.103	0.105	0.102	0.104	0.109	0.118	0.108	0.112	0.118	0.146	0.165	0.120	0.563	0.545	0.560
340.0	0.099	0.100	0.102	0.099	0.101	0.102	0.099	0.101	0.100	0.111	0.103	0.103	0.103	0.106	0.100	0.104	0.110	0.119	0.108	0.110	0.118	0.145	0.162	0.119	0.566	0.553	0.562
343.0	0.099	0.101	0.102	0.099	0.104	0.102	0.099	0.102	0.102	0.112	0.102	0.102	0.104	0.106	0.100	0.104	0.112	0.117	0.108	0.113	0.117	0.144	0.162	0.118	0.569	0.555	0.565
346.0	0.099	0.104	0.102	0.099	0.101	0.100	0.099	0.101	0.102	0.112	0.102	0.102	0.104	0.106	0.100	0.104	0.112	0.117	0.108	0.113	0.117	0.144	0.162	0.118	0.569	0.555	0.565
349.0	0.099	0.101	0.100	0.099	0.102	0.102	0.097	0.101	0.101	0.112	0.102	0.102	0.104	0.105	0.099	0.104	0.111	0.117	0.108	0.111	0.116	0.144	0.160	0.117	0.570	0.556	0.567

10^5 cfu/ml																										
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
100.0																										
130.0	0.086	0.092	0.089	0.087	0.094	0.091	0.091	0.097	0.094	0.101	0.108	0.105	0.105	0.114	0.131	0.101	0.114	0.114	0.101	0.114	0.104	0.114	0.124	0.102	0.105	0.102	0.117
192.0	0.081	0.085	0.085	0.083	0.086	0.086	0.086	0.090	0.090	0.097	0.101	0.101	0.115	0.113	0.111	0.113	0.112	0.114	0.113	0.112	0.114	0.123	0.122	0.124	0.121	0.121	0.123
195.0	0.083	0.085	0.086	0.085	0.086	0.088	0.088	0.090	0.091	0.099	0.100	0.102	0.113	0.111	0.111	0.114	0.114	0.115	0.114	0.114	0.115	0.124	0.124	0.125	0.125	0.125	0.129
198.0	0.082	0.083	0.086	0.083	0.085	0.087	0.087	0.088	0.091	0.098	0.099	0.102	0.111	0.110	0.111	0.116	0.115	0.118	0.116	0.115	0.118	0.125	0.125	0.127	0.129	0.129	0.131
201.0	0.084	0.084	0.086	0.085	0.086	0.087	0.089	0.089	0.091	0.099	0.100	0.102	0.110	0.110	0.108	0.118	0.119	0.121	0.118	0.119	0.121	0.128	0.128	0.131	0.133	0.134	0.136
204.0	0.081	0.083	0.086	0.082	0.084	0.087	0.086	0.088	0.091	0.097	0.098	0.102	0.111	0.109	0.107	0.121	0.121	0.123	0.121	0.121	0.123	0.130	0.130	0.133			
207.0	0.085	0.085	0.087	0.086	0.086	0.088	0.090	0.090	0.091	0.101	0.101	0.102	0.110	0.109	0.109	0.125	0.125	0.129	0.125	0.125	0.129	0.134	0.135	0.139			
210.0	0.082	0.083	0.087	0.083	0.084	0.088	0.087	0.087	0.092	0.098	0.098	0.102	0.113	0.113	0.113	0.129	0.129	0.131	0.129	0.129	0.131	0.138	0.139	0.141	0.153	0.154	0.156
213.0	0.081	0.082	0.086	0.082	0.083	0.087	0.086	0.087	0.091	0.097	0.097	0.101	0.111	0.110	0.111	0.123	0.124	0.126	0.133	0.134	0.136	0.142	0.143	0.145	0.164	0.164	0.165
216.0	0.081	0.084	0.086	0.082	0.086	0.088	0.086	0.089	0.091	0.096	0.100	0.102	0.110	0.110	0.108	0.134	0.134	0.135	0.144	0.144	0.145	0.153	0.153	0.155	0.171	0.168	0.177
223.0	0.083	0.088	0.087	0.084	0.089	0.088	0.088	0.093	0.091	0.099	0.104	0.102	0.113	0.113	0.113	0.143	0.149	0.132	0.168	0.176	0.156	0.187	0.186		0.174	0.172	0.174
226.0	0.084	0.086	0.084	0.085	0.087	0.085	0.088	0.090	0.089	0.099	0.101	0.100	0.115	0.113	0.111	0.145	0.148	0.131	0.172	0.179	0.157	0.193	0.194		0.190	0.183	0.189
229.0	0.084	0.086	0.086	0.085	0.088	0.088	0.089	0.091	0.091	0.099	0.102	0.102	0.113	0.111	0.111	0.143	0.146	0.130	0.174	0.184	0.160	0.199	0.204		0.197	0.190	0.197
232.0	0.083	0.086	0.087	0.085	0.087	0.088	0.088	0.091	0.091	0.099	0.101	0.102	0.111	0.110	0.111	0.142	0.141	0.130	0.174	0.186	0.160	0.204	0.210		0.203	0.198	0.206

235.0	0.081	0.085	0.085	0.083	0.086	0.086	0.086	0.090	0.090	0.097	0.101	0.101	0.110	0.110	0.108	0.139	0.141	0.127	0.176	0.189	0.160	0.210	0.222		0.212	0.205	0.215
238.0	0.083	0.085	0.086	0.085	0.086	0.088	0.088	0.090	0.091	0.099	0.100	0.102	0.111	0.109	0.107	0.141	0.141	0.125	0.177	0.188	0.159	0.214	0.228		0.223	0.214	0.221
241.0	0.082	0.083	0.086	0.083	0.085	0.087	0.087	0.088	0.091	0.098	0.099	0.102	0.110	0.109	0.109	0.138	0.135	0.125	0.175	0.184	0.160	0.215	0.231		0.232	0.222	0.231
244.0	0.084	0.084	0.086	0.085	0.086	0.087	0.089	0.089	0.091	0.099	0.100	0.102	0.108	0.108	0.109	0.137	0.134	0.124	0.174	0.185	0.161	0.216	0.237		0.241	0.231	0.242
247.0	0.081	0.083	0.086	0.082	0.084	0.087	0.086	0.088	0.091	0.097	0.098	0.102	0.106	0.108	0.106	0.134	0.134	0.118	0.174	0.187	0.159	0.218	0.226		0.250	0.241	0.252
250.0	0.085	0.085	0.087	0.086	0.086	0.088	0.090	0.090	0.091	0.101	0.101	0.102	0.110	0.108	0.106	0.136	0.136	0.118	0.176	0.188	0.156	0.223	0.228		0.263	0.250	0.263
253.0	0.082	0.083	0.087	0.083	0.084	0.088	0.087	0.087	0.092	0.098	0.098	0.102	0.107	0.107	0.107	0.132	0.130	0.117	0.168	0.185	0.154	0.213	0.229		0.271	0.259	0.274
256.0	0.081	0.082	0.086	0.082	0.083	0.087	0.086	0.087	0.091	0.097	0.097	0.101	0.106	0.106	0.106	0.130	0.126	0.113	0.163	0.181	0.152	0.211	0.228		0.280	0.269	0.283
259.0	0.081	0.084	0.086	0.082	0.086	0.088	0.086	0.089	0.091	0.096	0.100	0.102	0.105	0.106	0.106	0.124	0.124	0.111	0.157	0.181	0.147	0.207	0.235		0.290	0.279	0.295
262.0	0.083	0.082	0.087	0.085	0.083	0.088	0.088	0.087	0.091	0.099	0.097	0.102	0.106	0.106	0.106	0.131	0.129	0.117	0.153	0.175	0.140	0.206	0.233		0.302	0.290	0.305
265.0	0.082	0.083	0.087	0.084	0.084	0.089	0.087	0.087	0.092	0.098	0.098	0.103	0.106	0.106	0.106	0.130	0.128	0.119	0.147	0.172	0.134	0.199	0.229		0.312	0.299	0.315
268.0	0.082	0.083	0.085	0.083	0.084	0.087	0.087	0.087	0.090	0.098	0.098	0.101	0.106	0.105	0.107	0.126	0.125	0.119	0.140	0.169	0.130	0.196	0.226		0.321	0.310	0.329
271.0	0.081	0.081	0.087	0.082	0.082	0.088	0.086	0.086	0.091	0.096	0.097	0.102	0.105	0.105	0.106	0.122	0.119	0.115	0.131	0.163	0.125	0.189	0.216		0.330	0.320	0.339
274.0	0.082	0.082	0.088	0.083	0.083	0.089	0.087	0.087	0.093	0.097	0.098	0.104	0.106	0.105	0.106	0.119	0.116	0.114	0.126	0.156	0.118	0.185	0.214		0.341	0.330	0.348
277.0	0.081	0.081	0.092	0.083	0.083	0.093	0.086	0.086	0.097	0.097	0.097	0.107	0.106	0.104	0.106	0.116	0.116	0.114	0.118	0.147	0.115	0.176	0.209		0.349	0.339	0.358
280.0	0.082	0.083	0.088	0.083	0.084	0.089	0.087	0.088	0.093	0.097	0.098	0.103	0.106	0.105	0.107	0.114	0.113	0.115	0.113	0.137	0.114	0.169	0.202		0.358	0.350	0.369
283.0	0.082	0.085	0.090	0.083	0.086	0.091	0.087	0.090	0.095	0.097	0.101	0.105	0.105	0.107	0.106	0.112	0.112	0.117	0.107	0.128	0.112	0.159	0.191		0.365	0.357	0.380
286.0	0.081	0.083	0.090	0.083	0.085	0.091	0.086	0.088	0.095	0.097	0.099	0.105	0.105	0.106	0.106	0.112	0.113	0.114	0.107	0.122	0.111	0.153	0.185		0.373	0.367	0.388
289.0	0.082	0.085	0.092	0.083	0.086	0.094	0.087	0.090	0.097	0.097	0.100	0.108	0.105	0.107	0.106	0.111	0.113	0.115	0.104	0.117	0.109	0.144	0.178		0.384	0.375	0.398
292.0	0.082	0.084	0.091	0.083	0.085	0.093	0.087	0.089	0.096	0.097	0.100	0.107	0.105	0.107	0.107	0.111	0.114	0.115	0.103	0.115	0.110	0.136	0.176		0.394	0.388	0.410
295.0	0.083	0.085	0.092	0.084	0.087	0.094	0.088	0.090	0.097	0.098	0.101	0.108	0.105	0.107	0.108	0.111	0.114	0.116	0.103	0.111	0.110	0.130	0.171		0.402	0.396	0.419
298.0	0.082	0.085	0.091	0.083	0.086	0.092	0.087	0.090	0.096	0.097	0.101	0.107	0.107	0.108	0.105	0.112	0.114	0.114	0.104	0.109	0.109	0.124	0.157		0.413	0.405	0.430
301.0	0.082	0.084	0.094	0.083	0.085	0.095	0.087	0.089	0.098	0.098	0.100	0.109	0.106	0.107	0.106	0.111	0.114	0.116	0.103	0.108	0.108	0.118	0.150		0.423	0.415	0.440
304.0	0.083	0.086	0.096	0.084	0.087	0.098	0.087	0.091	0.101	0.098	0.102	0.112	0.105	0.107	0.107	0.111	0.113	0.118	0.102	0.108	0.108	0.114	0.145		0.431	0.424	0.449
307.0	0.082	0.085	0.093	0.083	0.087	0.094	0.087	0.090	0.098	0.097	0.101	0.109	0.105	0.108	0.107	0.111	0.114	0.116	0.102	0.109	0.109	0.112	0.139		0.439	0.434	0.459
310.0	0.082	0.083	0.090	0.083	0.084	0.092	0.087	0.088	0.095	0.097	0.099	0.106	0.105	0.107	0.106	0.111	0.115	0.114	0.104	0.109	0.106	0.113	0.132		0.451	0.445	0.468
313.0	0.082	0.086	0.095	0.083	0.087	0.096	0.087	0.090	0.100	0.098	0.101	0.110	0.105	0.107	0.107	0.110	0.116	0.116	0.103	0.107	0.106	0.112	0.128		0.457	0.454	0.478
316.0	0.083	0.087	0.093	0.084	0.088	0.095	0.088	0.092	0.098	0.099	0.102	0.109	0.106	0.107	0.109	0.111	0.117	0.118	0.103	0.109	0.107	0.111	0.121		0.464	0.461	0.486
319.0	0.082	0.085	0.094	0.084	0.086	0.095	0.087	0.090	0.098	0.098	0.100	0.109	0.104	0.107	0.107	0.111	0.114	0.115	0.103	0.110	0.108	0.112	0.128		0.472	0.468	0.496
322.0	0.083	0.086	0.095	0.084	0.087	0.096	0.088	0.090	0.100	0.099	0.101	0.110	0.106	0.108	0.107	0.112	0.115	0.114	0.105	0.109	0.109	0.113	0.122		0.478	0.475	0.500
325.0	0.082	0.089	0.097	0.084	0.090	0.098	0.087	0.093	0.102	0.098	0.104	0.112	0.106	0.107	0.107	0.111	0.114	0.115	0.105	0.108	0.107	0.114	0.118		0.484	0.482	0.508
328.0	0.083	0.091	0.096	0.084	0.092	0.097	0.087	0.096	0.101	0.098	0.106	0.112	0.104	0.108	0.107	0.110	0.116	0.115	0.104	0.109	0.108	0.114	0.111		0.490	0.487	0.515
331.0	0.083	0.089	0.094	0.085	0.091	0.096	0.088	0.094	0.099	0.099	0.105	0.110	0.105	0.108	0.106	0.111	0.116	0.115	0.104	0.109	0.110	0.113	0.106		0.495	0.493	0.522
334.0	0.083	0.088	0.094	0.084	0.090	0.095	0.088	0.093	0.099	0.098	0.104	0.110	0.105	0.107	0.107	0.110	0.116	0.115	0.104	0.109	0.109	0.113	0.104		0.500	0.499	0.528

337.0	**0.083**	**0.086**	**0.094**	**0.085**	**0.087**	**0.095**	**0.088**	**0.091**	**0.099**	**0.099**	**0.101**	**0.110**	**0.105**	**0.107**	**0.106**	**0.111**	**0.115**	**0.113**	**0.104**	**0.109**	**0.107**	**0.113**	**0.101**		**0.504**	**0.501**	**0.532**
340.0	**0.083**	**0.090**	**0.096**	**0.085**	**0.091**	**0.097**	**0.088**	**0.095**	**0.101**	**0.099**	**0.106**	**0.112**	**0.105**	**0.108**	**0.108**	**0.111**	**0.117**	**0.114**	**0.105**	**0.109**	**0.107**	**0.114**	**0.101**		**0.508**	**0.505**	**0.538**
343.0	**0.083**	**0.089**	**0.095**	**0.084**	**0.090**	**0.097**	**0.088**	**0.094**	**0.100**	**0.100**	**0.106**	**0.112**	**0.105**	**0.108**	**0.108**	**0.111**	**0.116**	**0.116**	**0.104**	**0.109**	**0.110**	**0.113**	**0.098**		**0.510**	**0.508**	**0.542**
346.0	**0.084**	**0.090**	**0.096**	**0.086**	**0.091**	**0.097**	**0.089**	**0.095**	**0.101**	**0.100**	**0.106**	**0.112**	**0.105**	**0.108**	**0.108**	**0.111**	**0.116**	**0.116**	**0.104**	**0.109**	**0.110**	**0.113**	**0.098**		**0.510**	**0.508**	**0.542**

5.2 Second publication article

Fluorometric detection of phages in liquid media: Application to turbid samples. This work has been published in the journal Analytica Chimica Acta.

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Fluorometric detection of phages in liquid media: Application to turbid samples

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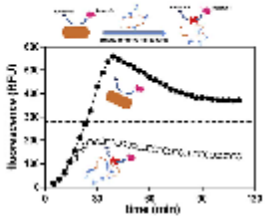
HIGHLIGHTS

- Detection of bacteriophages in turbid samples and in milk.
- The method does not require pre-treatment of the samples.
- Simple, rapid and sensitive.
- Suitable for miniaturization, automation and high-throughput analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

During the last years there has been a growing interest in the development of methods for phage detection and quantification in environmental, public health and industrial sectors. Good methods of phage monitoring contribute to progress in phage therapies, biocontrol and food safety studies. They have also been used to indicate the possible presence of microbiological hazards in drinking and recreational waters, and are an essential tool to prevent failure of microbe-based industrial bioreactors. Many of the sophisticated methods that have emerged to cover these needs are strongly hampered by the presence of turbidity in the samples, that results in decreased sensitivity. To avoid this, time consuming pretreatment steps must often be included that increase the overall complexity of the assays and the time required to perform them. With this in mind, we have explored an alternative method that fulfills the criteria of being simple, rapid and inexpensive and can be used to perform analysis in turbid media without any pretreatment steps. In this paper we develop a method that monitors lysis of an indicator culture when exposed to samples containing the target phage. The method is based on the properties of resazurin, a redox dye that becomes fluorescent when reduced by an active microbial culture. We analyzed the fluorescence kinetics of non-turbid phage-infected bacterial cultures as a function of both, phage abundance and initial cell concentration. For this purpose, different phage/host combinations were used and then, the addition of resazurin at different times (0, 30 and 60 min) was carefully evaluated for each phage/host combination, thus providing data for 168 combinations in total. Next, selected phage/host combinations were tested over 4 different turbidity models: 0, 1000, 2000 Nephelometric Turbidity Units (NTU) as well as in milk. The data obtained provided information about the duration of the assay and sensitivity thresholds in matrices with different turbidity grades. The results obtained indicate that the method can detect as few as 10 phage particles per assay volume within

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5.2.1 Second publication dataset

S2 dataset. This dataset contains the fluorescence (RFU) vs time data corresponding to 186 different phage/bacteria combinations used in the paper "Fluorometric detection of phages in liquid media: application to turbid samples" by Denis Rajnovic and Jordi Mas. Data have been used to create the graphs in Supplemental Fig. S2. A subset of the data has been used for Figures 9, 10, 11 and 12. Resazurin was added either at time 0, 30 or 60 min.

5x10^8 cfu/ml (0 min)																										
Time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
5	50	51	48	51	49	45	54	45	41	53	45	41													56	49	47
8	121	114	108	139	111	113	138	122	106	142	123	106													148	128	119
11	212	195	186	253	206	206	254	220	202	257	227	199													260	232	216
14	313	296	283	413	344	332	407	359	330	411	366	337													419	379	352
17	430	409	384	541	512	468	536	510	476	538	516	496													546	523	500
20	515	510	489	559	572	572	560	576	594	550	585	593													551	581	582
23	539	559	574	550	573	585	545	575	602	535	581	589													534	574	575
26	529	549	574	519	558	562	517	560	583	509	562	576													512	556	560
30	439	497	504	442	486	476	434	482	511	435	486	512													445	487	504
33	491	499	526	492	521	519	475	501	533	459	506	530													459	496	518
36	476	490	509	486	506	504	454	489	507	443	486	506													443	486	496
39	464	489	511	482	497	502	442	463	495	428	461	485													428	460	485
42	446	461	486	468	481	492	425	446	468	412	444	458													414	444	456
45	432	457	458	458	470	481	413	435	459	412	435	449													412	439	452
48	416	449	439	455	454	478	404	419	442	400	429	443													402	428	437
51	382	418	410	445	442	461	390	407	430	389	417	430													388	415	423
54	360	390	383	436	433	451	394	408	428	383	402	418													378	404	408
57	349	375	370	437	429	446	379	398	419	370	392	403													371	395	402

60	**322**	**339**	**339**	**431**	**417**	**436**	**370**	**383**	**404**	**359**	**385**	**389**												**357**	**381**	**389**
63	**308**	**320**	**326**	**426**	**417**	**431**	**359**	**375**	**400**	**352**	**375**	**382**												**350**	**374**	**380**
66	**301**	**310**	**318**	**422**	**413**	**423**	**355**	**377**	**391**	**354**	**366**	**383**												**347**	**365**	**374**
69	**294**	**294**	**307**	**415**	**411**	**424**	**344**	**362**	**383**	**344**	**352**	**368**												**342**	**346**	**365**
72	**288**	**286**	**300**	**416**	**411**	**421**	**340**	**363**	**376**	**326**	**341**	**357**												**326**	**342**	**349**
75	**287**	**284**	**297**	**418**	**407**	**425**	**341**	**358**	**374**	**323**	**341**	**355**												**322**	**336**	**347**
78	**281**	**273**	**292**	**409**	**405**	**416**	**323**	**340**	**365**	**323**	**333**	**353**												**323**	**336**	**348**
81	**278**	**268**	**290**	**408**	**401**	**416**	**327**	**339**	**361**	**319**	**332**	**350**												**317**	**333**	**344**
84	**280**	**269**	**290**	**408**	**403**	**414**	**321**	**338**	**363**	**320**	**334**	**356**												**319**	**331**	**349**
87	**276**	**262**	**287**	**408**	**400**	**414**	**326**	**338**	**364**	**319**	**334**	**347**												**317**	**333**	**349**
90	**274**	**260**	**287**	**405**	**402**	**416**	**320**	**333**	**364**	**320**	**327**	**348**												**315**	**325**	**344**
93	**280**	**262**	**291**	**409**	**404**	**418**	**322**	**338**	**360**	**320**	**330**	**347**												**317**	**322**	**344**
96	**278**	**260**	**288**	**408**	**408**	**421**	**325**	**339**	**363**	**321**	**330**	**347**												**321**	**325**	**346**
99	**279**	**259**	**289**	**406**	**407**	**420**	**319**	**336**	**365**	**318**	**331**	**348**												**316**	**328**	**345**
102	**282**	**262**	**295**	**410**	**410**	**428**	**321**	**336**	**368**	**321**	**329**	**348**												**315**	**323**	**344**
105	**281**	**261**	**293**	**406**	**410**	**431**	**323**	**338**	**367**	**319**	**339**	**350**												**315**	**327**	**345**
108	**281**	**261**	**293**	**407**	**412**	**429**	**320**	**334**	**362**	**322**	**335**	**346**												**317**	**327**	**343**
111	**286**	**264**	**298**	**411**	**410**	**433**	**331**	**351**	**373**	**322**	**338**	**355**												**317**	**340**	**352**

5x10^8 cfu/ml (30 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**
8	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**
11	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**
14	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**
17	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**
20	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**														**0**	**0**	**0**

23	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**												**0**	**0**	**0**
26	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**												**0**	**0**	**0**
30	**23**	**20**	**20**	**54**	**51**	**45**	**53**	**51**	**48**	**53**	**54**	**48**												**54**	**57**	**51**
33	**60**	**54**	**60**	**175**	**169**	**162**	**175**	**173**	**172**	**167**	**173**	**169**												**166**	**176**	**171**
36	**99**	**90**	**102**	**327**	**316**	**298**	**332**	**329**	**323**	**314**	**325**	**320**												**323**	**333**	**317**
39	**142**	**129**	**147**	**539**	**515**	**491**	**529**	**525**	**531**	**502**	**527**	**522**												**507**	**525**	**517**
42	**180**	**163**	**184**	**565**	**578**	**573**	**564**	**559**	**564**	**560**	**563**	**571**												**543**	**555**	**558**
45	**209**	**195**	**222**	**547**	**561**	**555**	**537**	**537**	**542**	**535**	**542**	**547**												**519**	**532**	**539**
48	**239**	**218**	**251**	**532**	**549**	**542**	**514**	**520**	**520**	**514**	**526**	**529**												**493**	**511**	**518**
51	**259**	**234**	**274**	**517**	**532**	**523**	**487**	**498**	**498**	**496**	**510**	**517**												**477**	**487**	**502**
54	**264**	**236**	**284**	**504**	**514**	**507**	**475**	**487**	**484**	**480**	**493**	**499**												**463**	**472**	**484**
57	**263**	**233**	**281**	**501**	**510**	**498**	**457**	**471**	**469**	**469**	**485**	**498**												**455**	**464**	**475**
60	**253**	**227**	**271**	**491**	**497**	**487**	**448**	**459**	**458**	**456**	**477**	**482**												**438**	**452**	**461**
63	**243**	**220**	**260**	**482**	**490**	**481**	**437**	**452**	**443**	**447**	**466**	**474**												**434**	**446**	**451**
66	**233**	**212**	**250**	**471**	**481**	**475**	**440**	**455**	**440**	**438**	**459**	**464**												**424**	**438**	**445**
69	**226**	**208**	**239**	**471**	**481**	**469**	**425**	**439**	**423**	**433**	**450**	**457**												**420**	**423**	**436**
72	**218**	**201**	**229**	**468**	**476**	**466**	**419**	**432**	**418**	**414**	**439**	**443**												**403**	**413**	**420**
75	**213**	**195**	**222**	**468**	**477**	**465**	**412**	**426**	**411**	**405**	**429**	**432**												**400**	**408**	**413**
78	**203**	**190**	**213**	**467**	**475**	**470**	**403**	**417**	**402**	**404**	**424**	**429**												**398**	**404**	**410**
81	**197**	**184**	**205**	**462**	**472**	**466**	**397**	**408**	**394**	**397**	**421**	**421**												**392**	**401**	**402**
84	**191**	**180**	**199**	**464**	**474**	**467**	**398**	**406**	**393**	**396**	**413**	**425**												**391**	**397**	**406**
87	**186**	**174**	**195**	**459**	**472**	**462**	**393**	**407**	**392**	**391**	**414**	**420**												**386**	**392**	**394**
90	**179**	**169**	**186**	**457**	**467**	**459**	**392**	**404**	**391**	**388**	**404**	**415**												**384**	**387**	**393**
93	**175**	**164**	**181**	**456**	**469**	**453**	**395**	**402**	**386**	**386**	**403**	**410**												**383**	**388**	**391**
96	**170**	**159**	**176**	**456**	**468**	**457**	**394**	**406**	**389**	**385**	**400**	**409**												**385**	**390**	**397**
99	**165**	**155**	**170**	**457**	**466**	**453**	**395**	**399**	**386**	**381**	**402**	**411**												**381**	**388**	**390**
102	**162**	**152**	**167**	**459**	**471**	**454**	**397**	**404**	**390**	**386**	**397**	**409**												**381**	**388**	**391**
105	**157**	**147**	**162**	**460**	**469**	**450**	**394**	**399**	**390**	**383**	**401**	**408**												**381**	**386**	**389**
108	**153**	**143**	**159**	**457**	**473**	**451**	**399**	**401**	**392**	**386**	**397**	**405**												**382**	**386**	**389**

111	**150**	**141**	**156**	**467**	**478**	**456**	**401**	**404**	**394**	**383**	**407**	**414**												**381**	**392**	**391**

5x10^8 cfu/ml (60 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
60	**39**	**38**	**37**	**165**	**193**	**154**	**340**	**309**	**305**																	**337**	**351**	**308**
63	**46**	**44**	**43**	**354**	**349**	**283**	**442**	**454**	**420**																	**576**	**548**	**483**
66	**52**	**50**	**48**	**455**	**454**	**411**	**467**	**462**	**495**																	**594**	**591**	**518**
69	**57**	**55**	**52**	**454**	**453**	**446**	**414**	**410**	**464**																	**535**	**541**	**512**
72	**64**	**60**	**57**	**432**	**431**	**432**	**389**	**384**	**446**																	**498**	**499**	**489**
75	**67**	**64**	**61**	**424**	**415**	**425**	**376**	**367**	**440**																	**475**	**479**	**484**
80	**81**	**75**	**71**	**362**	**356**	**388**	**279**	**261**	**393**																	**380**	**369**	**439**
85	**90**	**79**	**75**	**406**	**387**	**391**	**320**	**291**	**409**																	**404**	**401**	**449**
88	**96**	**84**	**79**	**407**	**379**	**387**	**316**	**287**	**399**																	**397**	**387**	**434**
91	**104**	**88**	**82**	**416**	**390**	**394**	**321**	**290**	**415**																	**393**	**393**	**433**
94	**106**	**91**	**85**	**412**	**391**	**392**	**315**	**284**	**405**																	**383**	**391**	**427**
97	**115**	**95**	**88**	**417**	**393**	**392**	**323**	**294**	**404**																	**390**	**390**	**428**
100	**118**	**97**	**92**	**418**	**391**	**388**	**319**	**298**	**395**																	**387**	**384**	**418**
103	**123**	**102**	**93**	**418**	**399**	**395**	**323**	**306**	**399**																	**393**	**391**	**423**
106	**126**	**105**	**97**	**417**	**393**	**394**	**331**	**305**	**400**																	**394**	**394**	**428**
109	**130**	**107**	**100**	**428**	**403**	**394**	**325**	**322**	**398**																	**387**	**390**	**417**
112	**135**	**111**	**102**	**425**	**405**	**392**	**333**	**317**	**408**																	**392**	**392**	**426**
115	**139**	**114**	**105**	**426**	**413**	**401**	**341**	**336**	**416**																	**405**	**407**	**434**
118	**142**	**117**	**107**	**431**	**416**	**402**	**349**	**343**	**421**																	**405**	**406**	**426**
121	**145**	**120**	**111**	**426**	**413**	**400**	**365**	**341**	**446**																	**426**	**427**	**452**
124	**149**	**122**	**113**	**425**	**414**	**401**	**374**	**340**	**449**																	**432**	**436**	**464**
127	**152**	**125**	**115**	**432**	**427**	**411**	**377**	**355**	**459**																	**444**	**446**	**481**
130	**155**	**127**	**118**	**427**	**424**	**411**	**378**	**344**	**453**																	**436**	**445**	**477**

133	**158**	**131**	**120**	**431**	**425**	**413**	**382**	**350**	**459**																**450**	**452**	**494**
136	**160**	**133**	**123**	**429**	**427**	**409**	**384**	**352**	**457**																**451**	**453**	**490**
139	**164**	**135**	**124**	**427**	**424**	**409**	**382**	**355**	**465**																**455**	**462**	**493**
142	**166**	**138**	**127**	**429**	**423**	**408**	**385**	**356**	**460**																**458**	**457**	**490**
145	**169**	**141**	**129**	**432**	**427**	**408**	**385**	**367**	**456**																**460**	**453**	**489**
148	**171**	**143**	**131**	**427**	**423**	**409**	**380**	**357**	**448**																**450**	**448**	**477**
151	**174**	**144**	**133**	**424**	**420**	**405**	**391**	**363**	**463**																**459**	**453**	**487**
154	**176**	**148**	**134**	**429**	**425**	**407**	**389**	**372**	**454**																**459**	**453**	**482**

2.5x10^8 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5	**15**	**15**	**16**	**13**	**13**		**13**	**12**	**13**	**14**	**14**	**13**													**14**	**14**	**14**
8	**32**	**32**	**34**	**30**	**28**		**28**	**27**	**27**	**29**	**30**	**29**													**31**	**32**	**32**
11	**56**	**56**	**62**	**57**	**54**		**52**	**48**	**51**	**52**	**53**	**51**													**59**	**63**	**67**
14	**85**	**85**	**98**	**93**	**93**		**92**	**78**	**83**	**83**	**89**	**83**													**99**	**109**	**117**
17	**118**	**118**	**131**	**135**	**135**		**135**	**121**	**125**	**124**	**132**	**124**													**145**	**157**	**166**
20	**143**	**145**	**161**	**177**	**178**		**186**	**167**	**170**	**174**	**186**	**174**													**199**	**211**	**222**
23	**171**	**173**	**190**	**220**	**219**		**242**	**218**	**218**	**238**	**247**	**226**													**253**	**268**	**279**
26	**182**	**184**	**207**	**261**	**261**		**288**	**265**	**266**	**298**	**302**	**282**													**311**	**324**	**341**
30	**179**	**186**	**210**	**344**	**335**		**379**	**350**	**356**	**390**	**403**	**387**													**425**	**424**	**440**
33	**183**	**191**	**215**	**392**	**386**		**435**	**401**	**410**	**473**	**489**	**459**													**477**	**488**	**505**
36	**177**	**186**	**209**	**427**	**420**		**480**	**447**	**455**	**519**	**531**	**500**													**521**	**534**	**550**
39	**178**	**184**	**210**	**472**	**458**		**525**	**501**	**503**	**561**	**556**	**541**													**554**	**555**	**567**
42	**177**	**181**	**204**	**501**	**497**		**541**	**537**	**537**	**557**	**535**	**537**													**544**	**546**	**553**
45	**174**	**179**	**201**	**528**	**526**		**529**	**550**	**547**	**537**	**509**	**519**													**527**	**532**	**542**
48	**170**	**175**	**194**	**548**	**556**		**523**	**549**	**538**	**526**	**497**	**502**													**515**	**521**	**533**
51	**166**	**170**	**193**	**540**	**552**		**507**	**529**	**519**	**515**	**479**	**479**													**501**	**508**	**521**

54	**161**	**167**	**185**	**531**	**543**		**499**	**521**	**505**	**500**	**471**	**473**													**491**	**494**	**508**
57	**160**	**166**	**185**	**533**	**543**		**493**	**506**	**497**	**497**	**461**	**461**													**480**	**488**	**495**
60	**156**	**161**	**177**	**527**	**535**		**483**	**495**	**483**	**483**	**451**	**451**													**463**	**466**	**476**
63	**153**	**157**	**174**	**522**	**533**		**477**	**485**	**473**	**478**	**443**	**441**													**453**	**458**	**462**
66	**149**	**154**	**171**	**523**	**533**		**470**	**479**	**466**	**471**	**445**	**442**													**442**	**447**	**452**
69	**146**	**150**	**168**	**519**	**527**		**464**	**467**	**461**	**460**	**437**	**434**													**426**	**438**	**440**
72	**143**	**147**	**165**	**519**	**522**		**458**	**463**	**457**	**443**	**422**	**420**													**413**	**417**	**423**
75	**141**	**145**	**163**	**514**	**521**		**451**	**457**	**448**	**440**	**417**	**411**													**407**	**410**	**416**
78	**139**	**144**	**160**	**513**	**521**		**437**	**439**	**437**	**432**	**413**	**407**													**399**	**404**	**411**
81	**135**	**139**	**157**	**508**	**513**		**430**	**437**	**431**	**424**	**409**	**401**													**392**	**393**	**403**
84	**136**	**139**	**155**	**511**	**515**		**424**	**431**	**424**	**419**	**403**	**397**													**388**	**391**	**396**
87	**133**	**136**	**153**	**508**	**504**		**420**	**425**	**420**	**413**	**398**	**389**													**379**	**380**	**387**
90	**130**	**135**	**151**	**498**	**503**		**415**	**418**	**416**	**408**	**393**	**385**													**379**	**380**	**388**
93	**129**	**133**	**149**	**503**	**505**		**411**	**416**	**410**	**407**	**392**	**386**													**374**	**379**	**384**
96	**126**	**132**	**148**	**494**	**494**		**407**	**413**	**412**	**401**	**386**	**380**													**372**	**374**	**380**
99	**125**	**130**	**146**	**488**	**493**		**409**	**409**	**407**	**399**	**386**	**378**													**370**	**374**	**374**
102	**125**	**130**	**146**	**494**	**496**		**407**	**409**	**406**	**398**	**383**	**374**													**369**	**369**	**377**
105	**124**	**127**	**144**	**481**	**488**		**409**	**411**	**410**	**398**	**385**	**375**													**371**	**368**	**376**
108	**122**	**127**	**145**	**479**	**483**		**405**	**409**	**408**	**392**	**381**	**374**													**366**	**367**	**374**
111	**122**	**127**	**144**	**478**	**482**		**412**	**414**	**413**	**396**	**389**	**380**													**369**	**370**	**378**

2.5x10^8 cfu/ml (30 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
5	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
8	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
11	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
14	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**

17	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
20	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
23	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
26	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**													**0**	**0**	**0**
30	**6**	**6**	**5**	**10**	**13**	**13**	**17**	**14**	**15**	**17**	**13**	**19**													**15**	**16**	**18**
33	**11**	**11**	**10**	**36**	**49**	**52**	**61**	**56**	**66**	**60**	**51**	**71**													**56**	**61**	**78**
36	**16**	**17**	**14**	**68**	**97**	**105**	**117**	**107**	**130**	**114**	**99**	**138**													**105**	**120**	**153**
39	**23**	**22**	**20**	**109**	**161**	**173**	**193**	**174**	**217**	**187**	**173**	**233**													**177**	**200**	**248**
42	**26**	**26**	**24**	**154**	**218**	**232**	**276**	**251**	**307**	**273**	**245**	**339**													**256**	**282**	**344**
45	**30**	**30**	**27**	**204**	**281**	**302**	**386**	**351**	**412**	**386**	**331**	**453**													**346**	**386**	**453**
48	**33**	**33**	**29**	**252**	**343**	**366**	**480**	**440**	**498**	**482**	**409**	**504**													**436**	**478**	**517**
51	**35**	**36**	**32**	**296**	**410**	**427**	**563**	**516**	**510**	**545**	**495**	**467**													**512**	**522**	**490**
54	**37**	**37**	**33**	**347**	**471**	**484**	**561**	**528**	**483**	**522**	**522**	**434**													**514**	**495**	**451**
57	**39**	**39**	**35**	**388**	**527**	**528**	**536**	**503**	**457**	**490**	**489**	**418**													**487**	**464**	**437**
60	**39**	**40**	**35**	**420**	**544**	**542**	**508**	**478**	**445**	**467**	**454**	**406**													**457**	**437**	**424**
63	**41**	**41**	**36**	**449**	**543**	**534**	**485**	**457**	**433**	**450**	**433**	**401**													**437**	**424**	**418**
66	**42**	**41**	**37**	**469**	**542**	**532**	**480**	**453**	**439**	**443**	**427**	**410**													**430**	**416**	**415**
69	**42**	**42**	**37**	**492**	**532**	**520**	**466**	**442**	**437**	**430**	**417**	**404**													**422**	**410**	**411**
72	**42**	**42**	**37**	**503**	**528**	**512**	**462**	**435**	**434**	**418**	**406**	**392**													**403**	**397**	**397**
75	**43**	**41**	**38**	**517**	**524**	**514**	**458**	**434**	**433**	**414**	**399**	**389**													**404**	**394**	**397**
78	**43**	**42**	**37**	**515**	**521**	**508**	**443**	**418**	**419**	**410**	**394**	**389**													**400**	**388**	**393**
81	**43**	**42**	**38**	**507**	**516**	**503**	**438**	**411**	**416**	**403**	**388**	**386**													**390**	**385**	**387**
84	**43**	**42**	**38**	**498**	**514**	**506**	**433**	**407**	**413**	**400**	**386**	**384**													**389**	**381**	**388**
87	**42**	**42**	**37**	**489**	**506**	**501**	**428**	**399**	**410**	**394**	**382**	**380**													**382**	**375**	**382**
90	**42**	**41**	**37**	**474**	**502**	**496**	**428**	**399**	**405**	**390**	**380**	**382**													**379**	**373**	**382**
93	**43**	**42**	**38**	**464**	**497**	**498**	**423**	**396**	**401**	**388**	**377**	**377**													**380**	**368**	**376**
96	**43**	**42**	**37**	**449**	**488**	**488**	**419**	**393**	**400**	**386**	**370**	**374**													**379**	**364**	**372**
99	**41**	**41**	**37**	**437**	**481**	**485**	**414**	**387**	**395**	**379**	**370**	**373**													**373**	**362**	**367**
102	**42**	**41**	**37**	**427**	**481**	**483**	**416**	**391**	**392**	**379**	**367**	**370**													**373**	**361**	**367**

105	**42**	**42**	**37**	**414**	**474**	**479**	**410**	**382**	**392**	**379**	**363**	**369**													**370**	**360**	**366**
108	**41**	**41**	**37**	**404**	**474**	**476**	**414**	**388**	**393**	**374**	**362**	**364**													**370**	**357**	**365**
111	**41**	**41**	**37**	**395**	**466**	**473**	**418**	**392**	**395**	**382**	**368**	**369**													**367**	**357**	**363**

2.5x10^8 cfu/ml (60 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
60	**6**	**5**	**5**	**62**	**68**	**69**	**107**	**115**	**113**																**118**	**115**	**117**
63	**10**	**9**	**8**	**133**	**143**	**140**	**211**	**232**	**220**																**261**	**254**	**241**
66	**14**	**13**	**12**	**206**	**212**	**213**	**309**	**332**	**325**																**392**	**374**	**347**
69	**18**	**17**	**15**	**309**	**324**	**320**	**500**	**507**	**497**																**560**	**521**	**476**
72	**22**	**20**	**18**	**407**	**426**	**441**	**549**	**502**	**495**																**547**	**521**	**486**
75	**24**	**22**	**20**	**444**	**466**	**484**	**540**	**486**	**481**																**522**	**506**	**478**
80	**34**	**30**	**27**	**539**	**525**	**509**	**468**	**425**	**450**																**484**	**477**	**449**
85	**40**	**32**	**30**	**558**	**547**	**502**	**478**	**437**	**458**																**492**	**485**	**467**
88	**44**	**36**	**32**	**557**	**544**	**498**	**469**	**429**	**452**																**489**	**483**	**464**
91	**49**	**38**	**35**	**558**	**543**	**494**	**475**	**437**	**457**																**483**	**480**	**462**
94	**51**	**40**	**36**	**556**	**541**	**487**	**466**	**439**	**452**																**472**	**466**	**454**
97	**56**	**43**	**39**	**553**	**542**	**488**	**467**	**444**	**451**																**473**	**470**	**452**
100	**58**	**45**	**41**	**550**	**537**	**481**	**461**	**442**	**451**																**463**	**459**	**444**
103	**62**	**48**	**42**	**552**	**536**	**485**	**466**	**445**	**452**																**468**	**459**	**445**
106	**64**	**50**	**45**	**555**	**537**	**483**	**468**	**445**	**450**																**461**	**452**	**441**
109	**67**	**51**	**46**	**548**	**536**	**481**	**464**	**443**	**450**																**464**	**458**	**436**
112	**70**	**54**	**48**	**546**	**534**	**477**	**463**	**439**	**448**																**452**	**446**	**436**
115	**72**	**56**	**50**	**549**	**536**	**478**	**467**	**442**	**448**																**460**	**452**	**435**
118	**75**	**58**	**51**	**549**	**535**	**476**	**463**	**441**	**444**																**454**	**449**	**425**
121	**77**	**60**	**54**	**547**	**532**	**476**	**463**	**439**	**450**																**461**	**443**	**438**
124	**79**	**61**	**55**	**549**	**532**	**475**	**464**	**438**	**448**																**456**	**444**	**435**

127	**81**	**63**	**56**	**544**	**536**	**475**	**464**	**440**	**448**																**453**	**445**	**430**
130	**83**	**64**	**58**	**546**	**530**	**474**	**461**	**439**	**446**																**451**	**445**	**429**
133	**85**	**67**	**60**	**545**	**533**	**475**	**461**	**440**	**452**																**463**	**450**	**436**
136	**86**	**68**	**61**	**544**	**533**	**474**	**461**	**437**	**451**																**454**	**446**	**429**
139	**89**	**70**	**63**	**544**	**533**	**475**	**461**	**440**	**453**																**468**	**455**	**443**
142	**90**	**72**	**65**	**541**	**531**	**474**	**463**	**441**	**453**																**463**	**445**	**441**
145	**92**	**74**	**66**	**541**	**531**	**471**	**463**	**443**	**456**																**462**	**451**	**442**
148	**94**	**75**	**67**	**539**	**526**	**469**	**463**	**445**	**457**																**461**	**456**	**440**
151	**95**	**75**	**68**	**538**	**528**	**470**	**465**	**447**	**460**																**473**	**459**	**451**
154	**97**	**78**	**69**	**538**	**530**	**470**	**467**	**450**	**462**																**468**	**461**	**449**

10^8 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
10	**33**	**31**	**29**	**34**	**34**	**32**	**38**	**35**	**33**	**37**	**35**	**33**	**37**	**35**	**32**	**38**	**34**	**33**							**37**	**34**	**33**
13	**46**	**42**	**39**	**46**	**45**	**46**	**49**	**49**	**44**	**50**	**50**	**45**	**50**	**49**	**44**	**50**	**48**	**44**							**52**	**46**	**43**
16	**57**	**52**	**50**	**57**	**56**	**56**	**63**	**61**	**60**	**63**	**63**	**59**	**64**	**60**	**58**	**64**	**60**	**60**							**65**	**59**	**54**
19	**67**	**63**	**60**	**71**	**69**	**68**	**75**	**75**	**81**	**78**	**79**	**79**	**78**	**75**	**74**	**79**	**74**	**78**							**82**	**75**	**68**
22	**71**	**68**	**67**	**82**	**79**	**80**	**91**	**88**	**98**	**93**	**93**	**100**	**94**	**89**	**90**	**95**	**88**	**95**							**100**	**90**	**82**
25	**71**	**71**	**71**	**91**	**88**	**90**	**106**	**106**	**115**	**110**	**112**	**122**	**110**	**105**	**112**	**113**	**104**	**113**							**119**	**108**	**99**
28	**72**	**72**	**72**	**97**	**93**	**97**	**123**	**123**	**132**	**127**	**128**	**140**	**126**	**120**	**129**	**130**	**120**	**128**							**139**	**126**	**117**
35	**67**	**69**	**69**	**105**	**105**	**107**	**159**	**152**	**160**	**163**	**156**	**172**	**161**	**149**	**155**	**166**	**155**	**160**							**169**	**156**	**146**
38	**68**	**67**	**69**	**107**	**109**	**112**	**172**	**169**	**177**	**184**	**173**	**191**	**177**	**167**	**171**	**186**	**171**	**176**							**190**	**174**	**161**
41	**67**	**68**	**67**	**110**	**112**	**115**	**192**	**188**	**194**	**208**	**196**	**211**	**199**		**187**	**208**	**190**	**193**							**215**	**196**	**179**
44	**67**	**67**	**67**	**117**	**118**	**121**	**220**	**211**	**217**	**239**	**219**	**238**	**229**	**214**	**210**	**240**	**215**	**220**							**248**	**224**	**206**
47	**65**	**66**	**66**	**121**	**120**	**122**	**258**	**236**	**241**	**276**	**250**	**270**	**265**	**241**	**235**	**281**	**246**	**252**							**291**	**255**	**239**
50	**65**	**65**	**65**	**128**	**126**	**126**	**291**	**265**	**269**	**325**	**283**	**305**	**305**	**274**	**273**	**328**	**282**	**285**							**331**	**293**	**274**
53	**63**	**63**	**63**	**133**	**131**	**130**	**345**	**302**	**302**	**370**	**325**	**350**	**354**	**314**	**305**	**380**	**321**	**328**							**363**	**340**	**317**

60	**60**	**60**	**61**	**142**	**140**	**137**	**419**	**385**	**372**	**384**	**416**	**445**	**382**	**394**	**402**	**392**	**418**	**436**							**417**	**409**
63	**59**	**58**	**61**	**143**	**142**	**138**	**408**	**416**	**408**	**370**	**439**	**484**	**369**	**418**	**429**	**394**	**444**	**482**							**422**	**443**
66	**59**	**59**	**61**	**146**	**142**	**141**	**409**	**444**	**443**	**368**	**455**	**523**	**361**	**431**	**466**	**393**	**444**	**513**							**410**	**459**
69	**58**	**57**	**61**	**149**	**145**	**141**	**421**	**453**	**480**	**375**	**457**	**539**	**377**	**426**	**491**	**406**	**438**	**522**							**403**	**458**
72	**58**	**56**	**59**	**149**	**145**	**142**	**434**	**460**	**480**	**396**	**454**	**528**	**400**	**429**	**492**	**428**	**436**	**506**							**410**	**451**
75	**58**	**55**	**59**	**154**	**146**	**143**	**456**	**475**	**494**	**420**	**453**	**521**	**427**	**434**	**484**	**438**	**430**	**499**							**415**	**420**	**449**
78	**57**	**54**	**58**	**154**	**146**	**144**	**461**	**482**	**488**	**432**	**455**	**508**	**444**	**447**	**480**	**444**	**432**	**491**							**439**	**431**	**438**
81	**57**	**54**	**58**	**155**	**148**	**144**	**467**	**495**	**490**	**438**	**453**	**503**	**454**	**450**	**473**	**439**	**429**	**484**							**453**	**440**	**436**
84	**56**	**53**	**57**	**154**	**147**	**143**	**462**	**492**	**483**	**436**	**446**	**492**	**444**	**448**	**466**	**433**	**425**	**479**							**461**	**441**	**425**
87	**56**	**52**	**56**	**154**	**147**	**143**	**463**	**493**	**480**	**437**	**438**	**487**	**448**	**449**	**460**	**431**	**421**	**475**							**464**	**439**	**424**
90	**56**	**52**	**56**	**155**	**146**	**141**	**458**	**490**	**478**	**426**	**435**	**485**	**438**	**445**	**455**	**426**	**418**	**469**							**456**	**434**	**418**
93	**56**	**50**	**56**	**155**	**144**	**141**	**452**	**484**	**471**	**426**	**432**	**480**	**436**	**446**	**452**	**419**	**413**	**463**							**446**	**431**	**410**
96	**56**	**51**	**55**	**154**	**145**	**141**	**453**	**486**	**472**	**417**	**427**	**474**	**426**	**440**	**445**	**415**	**409**	**461**							**438**	**422**	**399**
99	**55**	**50**	**55**	**154**	**143**	**139**	**445**	**479**	**464**	**410**	**424**	**469**	**420**	**433**	**440**	**408**	**403**	**461**							**431**	**420**	**392**
102	**55**	**49**	**55**	**154**	**141**	**137**	**442**	**478**	**465**	**406**	**418**	**468**	**417**	**428**	**432**	**405**	**401**	**450**							**425**	**414**	**390**
105	**55**	**48**	**54**	**151**	**138**	**135**	**429**	**467**	**454**	**399**	**410**	**461**	**413**	**426**	**429**	**398**	**397**	**450**							**423**	**411**	**389**
108	**54**	**48**	**54**	**152**	**136**	**135**	**427**	**464**	**452**	**394**	**406**	**460**	**409**	**422**	**427**	**395**	**393**	**443**							**416**	**407**	**381**
112	**55**	**48**	**54**	**152**	**136**	**133**	**427**	**466**	**456**	**394**	**410**	**460**	**404**	**420**	**423**	**391**	**392**	**444**							**412**	**404**	**381**
115	**54**	**47**	**54**	**149**	**134**	**131**	**427**	**464**	**452**	**398**	**405**	**455**	**410**	**418**	**420**	**395**	**391**	**443**							**407**	**404**	**386**
118	**55**	**46**	**54**	**150**	**132**	**129**	**424**	**462**	**451**	**386**	**407**	**458**	**395**	**413**	**417**	**386**	**389**	**441**							**404**	**402**	**377**
121	**55**	**46**	**54**	**150**	**132**	**129**	**424**	**462**	**451**	**386**	**407**	**458**	**395**	**413**	**417**	**386**	**389**	**441**							**404**	**402**	**377**
124	**55**	**46**	**54**	**150**	**132**	**129**	**424**	**462**	**451**	**386**	**407**	**458**	**395**	**413**	**417**	**386**	**389**	**441**							**404**	**402**	**377**
127	**55**	**46**	**54**	**150**	**132**	**129**	**424**	**462**	**451**	**386**	**407**	**458**	**395**	**413**	**417**	**386**	**389**	**441**							**404**	**402**	**377**
130	**55**	**46**	**54**	**150**	**132**	**129**	**424**	**462**	**451**	**386**	**407**	**458**	**395**	**413**	**417**	**386**	**389**	**441**							**404**	**402**	**377**

10^8 cfu/ml (30 min)																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							0	0	0
35	8	8	8	14	13	12	25	24	23	25	22	20	26	23	20	26	23	20							29	25	25
38	10	11	11	24	21	21	47	42	40	44	41	35	45	40	35	46	48	37							55	48	48
41	12	13	14	36	32	33	76	66	71	71	66	55	72		54	77	74	63							87	80	77
44	15	15	17	48	45	47	113	102	109	107	100	85	108	101	81	114	109	97							125	118	112
47	17	17	20	61	56	60	156	151	142	145	140	124	155	146	120	156	146	143							170	154	154
50	18	19	22	74	68	73	197	191	193	192	192	171	194	189	165	198	187	186							211	197	201
53	19	20	24	88	79	85	245	240	241	237	242	226	253	245	213	248	228	235							262	249	260
60	20	21	27	111	95	108	340	354	393	342	354	360	363	362	332	348	329	343							376	360	395
63	20	22	28	116	100	117	401	404	438	391	397	400	424	413	387	401	378	404							427	411	443
66	20	22	30	125	106	122	452	458	495	444	451	456	480	474	439	453	426	459							477	468	459
69	22	23	32	135	113	130	510	509	532	485	498	498	529	516	490	506	477	504							511	519	463
72	22	23	33	142	117	136	526	526	533	490	510	500	530	521	519	513	501	509							490	519	462
75	22	23	35	147	121	142	501	514	532	471	497	499	519	521	514	502	493	504							476	498	460
78	23	24	36	152	126	148	479	499	523	457	478	495	503	510	503	480	475	498							457	477	457
81	23	25	38	159	129	153	465	489	526	444	467	493	486	507	497	467	461	496							447	461	456
84	23	25	39	164	133	156	449	475	517	430	455	486	474	500	488	453	449	491							442	450	450
87	23	26	42	167	135	161	443	469	509	422	447	483	465	493	482	448	440	484							432	440	446
90	24	26	42	171	139	164	442	463	507	416	445	481	461	490	477	445	436	483							428	432	447
93	24	27	44	174	141	167	437	455	503	416	440	483	452	488	473	436	429	475							424	428	445
96	24	27	45	178	142	170	428	453	507	410	435	479	447	481	464	430	425	475							411	416	444

99	**24**	**28**	**47**	**178**	**144**	**172**	**423**	**447**	**499**	**405**	**431**	**476**	**442**	**477**	**464**	**424**	**417**	**467**							**406**	**409**	**440**
102	**25**	**28**	**49**	**181**	**145**	**176**	**424**	**445**	**496**	**401**	**432**	**477**	**440**	**474**	**455**	**421**	**416**	**473**							**401**	**410**	**437**
105	**25**	**29**	**49**	**183**	**145**	**175**	**418**	**437**	**488**	**394**	**424**	**468**	**432**	**473**	**453**	**418**	**410**	**466**							**395**	**402**	**434**
108	**25**	**29**	**50**	**185**	**148**	**178**	**414**	**434**	**482**	**389**	**419**	**468**	**427**	**472**	**451**	**412**	**407**	**464**							**387**	**398**	**434**
112	**26**	**30**	**52**	**187**	**149**	**179**	**416**	**439**	**493**	**389**	**422**	**468**	**428**	**471**	**448**	**413**	**407**	**464**							**390**	**396**	**434**
115	**26**	**30**	**53**	**189**	**149**	**181**	**411**	**429**	**486**	**388**	**416**	**472**	**434**	**471**	**448**	**410**	**405**	**465**							**391**	**393**	**434**
118	**27**	**31**	**55**	**191**	**150**	**181**	**413**	**434**	**495**	**386**	**419**	**471**	**424**	**469**	**446**	**409**	**401**	**465**							**387**	**388**	**435**

10^8 cfu/ml (60 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
13	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
16	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
19	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
22	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
25	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
28	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
35	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
38	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
41	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**							**0**	**0**	**0**
44	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
47	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
50	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
53	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
60	**8**	**6**	**6**	**13**	**12**	**12**	**51**	**46**	**40**	**60**	**51**	**50**	**59**	**57**	**50**	**62**	**57**	**53**							**8**	**10**	**8**
63	**11**	**7**	**7**	**20**	**18**	**20**	**91**	**87**	**86**	**110**	**98**	**105**	**109**	**104**	**101**	**119**	**106**	**102**							**69**	**65**	**61**
66	**14**	**8**	**8**	**32**	**29**	**32**	**147**	**148**	**137**	**172**	**160**	**169**	**170**	**167**	**165**	**186**	**175**	**171**							**131**	**127**	**129**

69	**16**	**9**	**9**	**44**	**44**	**47**	**216**	**215**	**213**	**241**	**238**	**244**	**242**	**245**	**248**	**277**	**260**	**247**							**206**	**204**	**212**
72	**19**	**10**	**10**	**52**	**53**	**55**	**293**	**302**	**287**	**321**	**329**	**328**	**332**	**345**	**328**	**367**	**364**	**343**							**293**	**287**	**299**
75	**22**	**11**	**11**	**60**	**59**	**63**	**376**	**390**	**363**	**410**	**415**	**414**	**418**	**432**	**410**	**453**	**459**	**427**							**394**	**386**	**403**
78	**25**	**11**	**12**	**66**	**65**	**69**	**450**	**460**	**429**	**485**	**482**	**494**	**491**	**497**	**481**	**504**	**502**	**496**							**486**	**483**	**493**
81	**28**	**12**	**13**	**73**	**70**	**75**	**507**	**499**	**488**	**507**	**502**	**515**	**517**	**504**	**499**	**510**	**506**	**509**							**521**	**519**	**521**
84	**30**	**13**	**13**	**77**	**74**	**80**	**513**	**493**	**492**	**497**	**498**	**501**	**508**	**502**	**488**	**503**	**499**	**501**							**520**	**519**	**512**
87	**32**	**13**	**13**	**82**	**78**	**84**	**513**	**494**	**489**	**487**	**498**	**492**	**500**	**504**	**472**	**503**	**501**	**490**							**511**	**514**	**494**
90	**34**	**13**	**14**	**87**	**80**	**86**	**507**	**486**	**484**	**478**	**497**	**478**	**495**	**500**	**456**	**501**	**498**	**479**							**514**	**507**	**478**
93	**37**	**14**	**15**	**90**	**83**	**88**	**502**	**483**	**478**	**470**	**493**	**472**	**490**	**497**	**448**	**498**	**492**	**474**							**501**	**500**	**460**
96	**39**	**14**	**15**	**93**	**85**	**89**	**500**	**491**	**479**	**463**	**493**	**465**	**485**	**494**	**439**	**488**	**490**	**463**							**499**	**493**	**452**
99	**41**	**14**	**16**	**95**	**85**	**89**	**495**	**486**	**473**	**457**	**491**	**453**	**470**	**493**	**429**	**487**	**487**	**453**							**495**	**487**	**443**
102	**43**	**15**	**16**	**95**	**87**	**89**	**498**	**488**	**468**	**452**	**491**	**453**	**473**	**489**	**423**	**489**	**487**	**448**							**488**	**483**	**436**
105	**45**	**15**	**16**	**97**	**87**	**89**	**487**	**483**	**458**	**446**	**488**	**442**	**466**	**485**	**414**	**483**	**487**	**437**							**483**	**478**	**429**
108	**47**	**16**	**17**	**98**	**88**	**88**	**486**	**482**	**463**	**442**	**487**	**434**	**460**	**484**	**412**	**481**	**483**	**436**							**480**	**472**	**422**
112	**49**	**15**	**18**	**101**	**90**	**89**	**490**	**487**	**464**	**440**	**488**	**433**	**462**	**483**	**408**	**481**	**484**	**433**							**474**	**471**	**416**
115	**51**	**16**	**18**	**102**	**89**	**88**	**483**	**479**	**458**	**442**	**486**	**429**	**457**	**481**	**403**	**482**	**480**	**433**							**476**	**469**	**417**
118	**54**	**16**	**18**	**103**	**89**	**87**	**487**	**486**	**461**	**436**	**489**	**435**	**454**	**484**	**407**	**480**	**481**	**430**							**475**	**466**	**410**

5x10^7 cfu/ml (0 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
10	**17**	**14**	**14**	**17**	**16**	**17**	**18**	**16**	**18**	**17**	**16**	**18**	**17**	**14**	**17**	**18**	**16**	**17**							**18**	**18**	**18**
13	**22**	**19**	**19**	**23**	**20**	**24**	**24**	**23**	**25**	**23**	**22**	**24**	**23**	**19**	**24**	**25**	**21**	**24**							**24**	**24**	**24**
16	**28**	**24**	**24**	**29**	**26**	**30**	**31**	**28**	**31**	**30**	**28**	**31**	**30**	**24**	**31**	**31**	**27**	**31**							**32**	**30**	**32**
19	**32**	**29**	**27**	**34**	**31**	**36**	**36**	**33**	**38**	**36**	**34**	**38**	**36**	**29**	**38**	**37**	**33**	**37**							**39**	**36**	**38**
22	**33**	**30**	**30**	**38**	**34**	**40**	**41**	**38**	**44**	**40**	**38**	**44**	**40**	**32**	**43**	**41**	**37**	**43**							**44**	**41**	**44**
25	**34**	**31**	**31**	**41**	**38**	**43**	**45**	**42**	**49**	**44**	**42**	**49**	**45**	**35**	**49**	**46**	**41**	**47**							**48**	**45**	**48**
28	**34**	**32**	**32**	**43**	**39**	**45**	**48**	**44**	**53**	**47**	**45**	**53**	**48**	**37**	**52**	**48**	**44**	**51**							**51**	**48**	**51**

35	**34**	**33**	**32**	**44**	**43**	**48**	**52**	**50**	**59**	**52**	**49**	**61**	**51**	**41**	**62**	**54**	**49**	**59**							**55**	**57**	**58**
38	**34**	**31**	**31**	**43**	**42**	**47**	**54**	**51**	**63**	**55**	**52**	**66**	**54**	**42**	**65**	**57**	**53**	**64**							**56**	**61**	**63**
41	**33**	**32**	**30**	**44**	**41**	**48**	**56**	**54**	**67**	**59**	**55**	**70**	**57**		**69**	**61**	**57**	**69**							**62**	**67**	**69**
44	**35**	**32**	**31**	**46**	**43**	**48**	**61**	**57**	**74**	**65**	**59**	**77**	**64**	**48**	**76**	**69**	**65**	**77**							**68**	**76**	**76**
47	**33**	**32**	**30**	**46**	**41**	**48**	**66**	**61**	**81**	**73**	**64**	**84**	**69**	**52**	**84**	**78**	**71**	**86**							**76**	**86**	**85**
50	**34**	**32**	**30**	**47**	**43**	**49**	**75**	**67**	**91**	**83**	**72**	**96**	**79**	**58**	**93**	**89**	**83**	**98**							**87**	**98**	**96**
53	**33**	**32**	**30**	**47**	**42**	**50**	**83**	**75**	**100**	**94**	**81**	**107**	**90**	**65**	**106**	**102**	**93**	**110**							**99**	**112**	**109**
56	**34**	**32**	**29**	**47**	**43**	**49**	**104**	**92**	**126**	**121**	**104**	**136**	**117**	**82**	**132**	**134**	**128**	**139**							**131**	**148**	**144**
60	**33**	**32**	**28**	**47**	**42**	**49**	**113**	**98**	**135**	**136**	**112**	**150**	**131**	**90**	**145**	**150**	**140**	**153**							**144**	**165**	**159**
63	**33**	**32**	**28**	**48**	**42**	**50**	**126**	**107**	**146**	**153**	**124**	**165**	**149**	**101**	**159**	**171**	**158**	**172**							**164**	**185**	**180**
66	**34**	**33**	**29**	**49**	**43**	**50**	**142**	**120**	**166**	**180**	**143**	**185**	**173**	**114**	**180**	**200**	**185**	**198**							**194**	**215**	**208**
69	**35**	**32**	**29**	**49**	**42**	**50**	**161**	**134**	**188**	**209**	**165**	**212**	**204**	**132**	**207**	**237**	**219**	**229**							**228**	**252**	**242**
72	**34**	**34**	**28**	**50**	**43**	**50**	**185**	**150**	**214**	**241**	**192**	**241**	**233**	**153**	**233**	**272**	**254**	**262**							**263**	**291**	**280**
75	**35**	**33**	**28**	**50**	**42**	**50**	**207**	**169**	**242**	**271**	**222**	**276**	**260**	**177**	**264**	**301**	**297**	**299**							**283**	**333**	**325**
78	**35**	**33**	**28**	**51**	**42**	**50**	**235**	**192**	**273**	**284**	**258**	**313**	**257**	**209**	**292**	**309**	**356**	**345**							**278**	**382**	**378**
81	**35**	**34**	**28**	**51**	**43**	**51**	**258**	**212**	**305**	**282**	**295**	**350**	**249**	**237**	**321**	**309**	**419**	**392**							**276**	**422**	**428**
84	**35**	**33**	**28**	**52**	**43**	**51**	**281**	**232**	**339**	**288**	**332**	**389**	**249**	**271**	**338**	**319**	**479**	**439**							**285**	**454**	**473**
87	**36**	**34**	**28**	**52**	**43**	**50**	**301**	**251**	**373**	**294**	**363**	**418**	**261**	**308**	**338**	**341**	**502**	**480**							**301**	**468**	**490**
90	**35**	**34**	**28**	**52**	**42**	**50**	**315**	**268**	**412**	**318**	**386**	**445**	**283**	**331**	**339**	**371**	**494**	**479**							**331**	**458**	**479**
93	**36**	**35**	**28**	**53**	**43**	**50**	**330**	**285**	**442**	**344**	**408**	**466**	**307**	**343**	**356**	**401**	**497**	**472**							**358**	**447**	**468**
96	**36**	**34**	**27**	**53**	**43**	**50**	**338**	**298**	**472**	**374**	**428**	**466**	**337**	**358**	**370**	**412**	**494**	**452**							**392**	**436**	**458**
99	**37**	**35**	**28**	**54**	**43**	**50**	**341**	**308**	**503**	**409**	**448**	**460**	**368**	**383**	**397**	**408**	**488**	**447**							**421**	**429**	**453**
102	**36**	**35**	**27**	**54**	**42**	**50**	**341**	**312**	**512**	**426**	**452**	**451**	**392**	**409**	**411**	**404**	**486**	**441**							**431**	**421**	**444**
105	**37**	**35**	**28**	**54**	**42**	**50**	**341**	**314**	**521**	**436**	**449**	**444**	**407**	**421**	**420**	**398**	**485**	**435**							**433**	**412**	**438**
108	**38**	**36**	**28**	**55**	**43**	**50**	**339**	**315**	**530**	**441**	**450**	**443**	**414**	**419**	**421**	**393**	**487**	**433**							**432**	**409**	**434**
112	**38**	**36**	**28**	**54**	**43**	**50**	**328**	**308**	**519**	**437**	**440**	**437**	**410**	**413**	**417**	**387**	**475**	**420**							**424**	**406**	**430**
115	**36**	**34**	**26**	**54**	**41**	**48**	**323**	**304**	**522**	**433**	**432**	**430**	**404**	**400**	**408**	**378**	**472**	**418**							**416**	**399**	**419**
118	**36**	**34**	**26**	**54**	**41**	**48**	**323**	**304**	**522**	**433**	**432**	**430**	**404**	**400**	**408**	**378**	**472**	**418**							**416**	**399**	**419**
122	**36**	**34**	**26**	**54**	**41**	**48**	**323**	**304**	**522**	**433**	**432**	**430**	**404**	**400**	**408**	**378**	**472**	**418**							**416**	**399**	**419**

126	**36**	**34**	**25**	**54**	**41**	**48**	**323**	**304**	**522**	**433**	**432**	**430**	**404**	**400**	**408**	**378**	**472**	**418**							**416**	**398**	**419**
130	**36**	**34**	**25**	**54**	**41**	**48**	**323**	**304**	**522**	**433**	**432**	**430**	**404**	**399**	**408**	**378**	**472**	**418**							**416**	**398**	**419**
134	**36**	**34**	**25**	**53**	**41**	**47**	**322**	**303**	**522**	**433**	**431**	**430**	**404**	**399**	**408**	**377**	**472**	**417**							**416**	**398**	**419**
138	**35**	**34**	**25**	**53**	**40**	**47**	**322**	**303**	**521**	**432**	**431**	**429**	**404**	**399**	**408**	**377**	**471**	**417**							**415**	**398**	**419**
142	**35**	**33**	**25**	**53**	**40**	**47**	**322**	**303**	**521**	**432**	**431**	**429**	**403**	**399**	**407**	**377**	**471**	**417**							**415**	**398**	**418**

5x10^7 cfu/ml (30 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
10	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
13	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
16	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
19	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
22	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
25	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
28	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
35	**5**	**5**	**5**	**6**	**7**	**6**	**10**	**10**	**9**	**11**	**11**	**10**	**10**	**11**	**9**	**12**	**11**	**10**							**12**	**12**	**12**
38	**6**	**6**	**6**	**8**	**8**	**8**	**15**	**15**	**12**	**16**	**17**	**15**	**15**	**17**	**14**	**18**	**17**	**16**							**19**	**21**	**19**
41	**7**	**7**	**7**	**10**	**10**	**10**	**21**	**21**	**17**	**21**	**23**	**22**	**21**		**19**	**26**	**26**	**22**							**29**	**30**	**27**
44	**7**	**8**	**7**	**12**	**12**	**12**	**28**	**29**	**23**	**29**	**30**	**30**	**28**	**32**	**27**	**38**	**39**	**32**							**41**	**43**	**39**
47	**8**	**8**	**8**	**14**	**15**	**14**	**38**	**40**	**29**	**39**	**40**	**43**	**37**	**43**	**37**	**55**	**56**	**43**							**55**	**55**	**52**
50	**8**	**9**	**8**	**15**	**16**	**16**	**48**	**53**	**37**	**51**	**53**	**54**	**49**	**60**	**49**	**69**	**69**	**56**							**67**	**69**	**65**
53	**9**	**9**	**9**	**17**	**17**	**18**	**59**	**65**	**43**	**71**	**75**	**67**	**63**	**81**	**62**	**84**	**83**	**71**							**83**	**83**	**82**
56	**9**	**10**	**10**	**18**	**19**	**19**	**84**	**90**	**64**	**111**	**127**	**102**	**102**	**125**	**99**	**118**	**118**	**109**							**118**	**117**	**126**
60	**10**	**10**	**10**	**19**	**20**	**20**	**92**	**102**	**66**	**132**	**149**	**112**	**113**	**136**	**106**	**130**	**129**	**120**							**134**	**133**	**140**
63	**10**	**10**	**10**	**19**	**20**	**21**	**102**	**112**	**73**	**144**	**164**	**124**	**125**	**150**	**118**	**145**	**143**	**134**							**151**	**149**	**162**
66	**10**	**11**	**11**	**20**	**21**	**22**	**115**	**124**	**83**	**165**	**185**	**141**	**142**	**170**	**132**	**168**	**162**	**155**							**175**	**174**	**190**
69	**10**	**11**	**11**	**20**	**21**	**22**	**128**	**140**	**92**	**188**	**210**	**162**	**162**	**195**	**151**	**196**	**189**	**180**							**205**	**201**	**226**

72	**11**	**11**	**11**	**20**	**22**	**23**	**146**	**158**	**103**	**219**	**245**	**186**	**188**	**223**	**173**	**224**	**216**	**209**							**238**	**233**	**266**
75	**11**	**11**	**11**	**21**	**22**	**23**	**164**	**178**	**115**	**255**	**276**	**211**	**216**	**252**	**201**	**257**	**246**	**245**							**277**	**270**	**315**
78	**11**	**11**	**12**	**21**	**22**	**24**	**187**	**201**	**132**	**293**	**314**	**242**	**251**	**292**	**230**	**294**	**279**	**291**							**322**	**308**	**376**
81	**10**	**11**	**12**	**21**	**23**	**23**	**207**	**222**	**145**	**332**	**361**	**274**	**282**	**328**	**264**	**336**	**316**	**340**							**369**	**354**	**439**
84	**11**	**12**	**12**	**21**	**23**	**24**	**230**	**244**	**160**	**379**	**408**	**308**	**319**	**371**	**302**	**374**	**359**	**398**							**408**	**393**	**497**
87	**11**	**12**	**12**	**22**	**23**	**24**	**250**	**265**	**179**	**423**	**456**	**349**	**351**	**398**	**343**	**401**	**389**	**460**							**432**	**417**	**508**
90	**11**	**12**	**12**	**21**	**23**	**24**	**272**	**289**	**197**	**465**	**485**	**372**	**363**	**417**	**382**	**409**	**399**	**484**							**439**	**424**	**507**
93	**11**	**12**	**13**	**21**	**23**	**24**	**288**	**306**	**214**	**482**	**491**	**387**	**368**	**424**	**407**	**416**	**416**	**485**							**440**	**429**	**504**
96	**11**	**12**	**13**	**21**	**23**	**24**	**306**	**328**	**226**	**473**	**478**	**397**	**373**	**436**	**423**	**425**	**424**	**481**							**430**	**421**	**501**
99	**11**	**13**	**13**	**21**	**23**	**24**	**320**	**345**	**238**	**459**	**470**	**408**	**390**	**424**	**410**	**421**	**428**	**476**							**420**	**414**	**499**
102	**11**	**12**	**13**	**21**	**23**	**24**	**327**	**355**	**246**	**449**	**457**	**419**	**406**	**412**	**395**	**417**	**423**	**473**							**409**	**401**	**494**
105	**12**	**13**	**13**	**21**	**23**	**24**	**332**	**364**	**254**	**438**	**451**	**418**	**410**	**410**	**386**	**411**	**415**	**470**							**403**	**395**	**495**
108	**12**	**13**	**14**	**22**	**24**	**25**	**340**	**374**	**260**	**442**	**456**	**416**	**407**	**406**	**379**	**407**	**408**	**471**							**397**	**390**	**496**
112	**12**	**13**	**14**	**22**	**23**	**24**	**334**	**371**	**265**	**437**	**446**	**415**	**405**	**398**	**373**	**397**	**404**	**466**							**394**	**387**	**491**
115	**10**	**11**	**12**	**19**	**21**	**22**	**338**	**377**	**267**	**434**	**442**	**411**	**396**	**389**	**364**	**393**	**400**	**460**							**385**	**382**	**487**
118	**10**	**11**	**12**	**19**	**21**	**22**	**338**	**377**	**267**	**434**	**442**	**411**	**396**	**389**	**364**	**393**	**400**	**460**							**385**	**382**	**487**
122	**10**	**11**	**12**	**19**	**21**	**22**	**338**	**377**	**267**	**434**	**442**	**411**	**396**	**389**	**364**	**393**	**400**	**460**							**385**	**382**	**487**
126	**10**	**11**	**12**	**19**	**21**	**22**	**338**	**377**	**267**	**434**	**442**	**410**	**396**	**389**	**364**	**393**	**400**	**460**							**385**	**382**	**487**
130	**10**	**11**	**11**	**19**	**21**	**22**	**338**	**377**	**267**	**434**	**442**	**410**	**396**	**389**	**364**	**393**	**400**	**460**							**385**	**382**	**487**
134	**9**	**11**	**11**	**19**	**20**	**22**	**337**	**377**	**267**	**434**	**442**	**410**	**396**	**389**	**364**	**393**	**399**	**460**							**385**	**381**	**487**
138	**9**	**10**	**11**	**19**	**20**	**21**	**337**	**376**	**267**	**433**	**441**	**410**	**395**	**388**	**364**	**392**	**399**	**459**							**384**	**381**	**487**
142	**9**	**10**	**11**	**18**	**20**	**21**	**337**	**376**	**266**	**433**	**441**	**410**	**395**	**388**	**363**	**392**	**399**	**459**							**384**	**381**	**486**

5x10^7 cfu/ml (60 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
13	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
16	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**

19	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
22	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
25	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
28	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
35	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
38	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
41	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
44	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**							**0**	**0**	**0**
47	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
50	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
53	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
56	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**							**0**	**0**	**0**
60	**6**	**5**	**4**	**5**	**6**	**5**	**18**	**18**	**17**	**24**	**21**	**18**	**25**	**21**	**19**	**24**	**23**	**22**							**28**	**24**	**22**
63	**6**	**7**	**5**	**6**	**7**	**6**	**28**	**29**	**25**	**41**	**36**	**29**	**40**	**36**	**36**	**39**	**37**	**36**							**44**	**42**	**42**
66	**7**	**7**	**5**	**7**	**8**	**7**	**40**	**43**	**39**	**63**	**52**	**46**	**59**	**58**	**55**	**62**	**58**	**59**							**74**	**70**	**70**
69	**8**	**9**	**6**	**9**	**10**	**9**	**59**	**64**	**58**	**92**	**74**	**73**	**89**	**84**	**85**	**93**	**84**	**89**							**104**	**101**	**103**
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78	**11**	**11**	**7**	**11**	**14**	**11**	**138**	**156**	**140**	**189**	**175**	**172**	**192**	**181**	**182**	**198**	**185**	**188**							**215**	**208**	**216**
81	**12**	**12**	**7**	**12**	**15**	**12**	**164**	**187**	**168**	**227**	**210**	**206**	**231**	**220**	**222**	**240**	**223**	**230**							**263**	**255**	**266**
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87	**13**	**14**	**8**	**13**	**16**	**13**	**216**	**250**	**223**	**301**	**295**	**281**	**325**	**305**	**303**	**332**	**311**	**323**							**376**	**358**	**380**
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108	**19**	**21**	**9**	**14**	**21**	**15**	**340**	**433**	**366**	**423**	**442**	**432**	**412**	**393**	**393**	**399**	**399**	**405**							**409**	**415**	**440**

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118	**20**	**21**	**7**	**13**	**20**	**13**	**349**	**473**	**395**	**433**	**434**	**422**	**400**	**397**	**384**	**381**	**386**	**387**							**403**	**402**	**413**
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2.5x10^7 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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87				**37**	**27**	**34**	**83**	**80**	**83**	**217**	**173**	**155**	**263**	**198**	**167**	**261**	**189**	**164**	**256**	**189**	**151**	**289**	**200**	**159**	**377**	**275**	**255**
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93				**37**	**27**	**35**	**88**	**87**	**92**	**287**	**230**	**212**	**369**	**278**	**234**	**366**	**267**	**229**	**360**	**268**	**215**	**406**	**283**	**230**	**464**	**390**	**364**
96				**38**	**28**	**35**	**93**	**91**	**97**	**327**	**266**	**245**	**423**	**326**	**277**	**413**	**316**	**272**	**424**	**318**	**254**	**456**	**336**	**273**	**460**	**460**	**430**
99				**39**	**28**	**36**	**94**	**93**	**101**	**362**	**300**	**278**	**448**	**381**	**323**	**431**	**368**	**320**	**458**	**377**	**300**	**466**	**399**	**323**	**459**	**490**	**482**
102				**38**	**28**	**36**	**95**	**96**	**103**	**397**	**335**	**314**	**453**	**440**	**378**	**431**	**423**	**373**	**460**	**439**	**354**	**461**	**457**	**385**	**455**	**486**	**485**
105				**40**	**28**	**37**	**98**	**99**	**108**	**425**	**379**	**352**	**452**	**481**	**434**	**431**	**455**	**433**	**459**	**481**	**415**	**461**	**480**	**452**	**454**	**485**	**478**
108				**39**	**28**	**36**	**99**	**101**	**108**	**436**	**406**	**386**	**449**	**485**	**474**	**431**	**458**	**465**	**458**	**490**	**468**	**458**	**481**	**484**	**453**	**483**	**480**
111				**39**	**28**	**36**	**100**	**102**	**111**	**441**	**438**	**422**	**449**	**484**	**482**	**427**	**454**	**468**	**454**	**487**	**483**	**456**	**479**	**487**	**452**	**480**	**474**
114				**40**	**29**	**38**	**103**	**104**	**113**	**447**	**459**	**446**	**448**	**482**	**482**	**428**	**453**	**468**	**452**	**483**	**478**	**454**	**475**	**484**	**450**	**478**	**473**
117				**40**	**29**	**37**	**102**	**104**	**114**	**444**	**471**	**462**	**448**	**480**	**478**	**429**	**451**	**464**	**451**	**482**	**478**	**455**	**471**	**480**	**449**	**476**	**474**
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123				**41**	**29**	**38**	**105**	**107**	**116**	**444**	**482**	**477**	**447**	**481**	**475**	**422**	**446**	**455**	**446**	**477**	**474**	**448**	**470**	**477**	**441**	**470**	**465**
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132				**42**	**30**	**39**	**107**	**111**	**118**	**445**	**483**	**478**	**446**	**482**	**478**	**418**	**440**	**455**	**442**	**471**	**464**	**444**	**464**	**471**	**438**	**462**	**459**
135				**42**	**29**	**39**	**108**	**111**	**119**	**440**	**482**	**480**	**443**	**481**	**478**	**423**	**442**	**452**	**442**	**470**	**463**	**445**	**464**	**473**	**438**	**465**	**461**

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141				**43**	**30**	**40**	**110**	**112**	**122**	**441**	**482**	**476**	**442**	**477**	**477**	**420**	**439**	**447**	**435**	**465**	**458**	**443**	**459**	**466**	**435**	**456**	**457**
144				**42**	**30**	**39**	**110**	**113**	**121**	**434**	**482**	**478**	**443**	**481**	**477**	**419**	**438**	**448**	**436**	**464**	**457**	**440**	**459**	**467**	**432**	**457**	**456**
147				**42**	**30**	**39**	**110**	**112**	**122**	**437**	**479**	**475**	**441**	**479**	**477**	**422**	**442**	**452**	**435**	**463**	**455**	**440**	**461**	**466**	**430**	**454**	**456**

2.5x10^7 cfu/ml (30 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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63				**13**	**7**	**8**	**36**	**27**	**26**	**50**	**35**	**38**	**54**	**45**	**47**	**54**	**46**	**46**	**52**	**53**	**51**	**55**	**58**	**54**	**54**	**58**	**56**
66				**14**	**7**	**8**	**39**	**28**	**28**	**58**	**38**	**42**	**59**	**50**	**54**	**61**	**53**	**51**	**59**	**59**	**57**	**63**	**67**	**64**	**66**	**68**	**66**
69				**15**	**7**	**8**	**44**	**31**	**30**	**66**	**44**	**47**	**69**	**57**	**60**	**70**	**59**	**57**	**69**	**68**	**64**	**74**	**78**	**73**	**80**	**83**	**81**
72				**16**	**7**	**9**	**47**	**32**	**31**	**76**	**49**	**54**	**82**	**65**	**68**	**81**	**67**	**62**	**80**	**80**	**76**	**85**	**92**	**86**	**97**	**102**	**98**
75				**16**	**7**	**8**	**51**	**35**	**33**	**88**	**56**	**62**	**93**	**74**	**78**	**92**	**76**	**71**	**93**	**93**	**88**	**102**	**106**	**102**	**105**	**108**	**106**
78				**17**	**8**	**9**	**56**	**37**	**36**	**104**	**64**	**71**	**112**	**86**	**89**	**111**	**87**	**81**	**111**	**110**	**104**	**119**	**127**	**118**	**132**	**136**	**131**
81				**17**	**7**	**9**	**60**	**41**	**38**	**120**			**132**	**100**	**104**	**129**	**100**	**94**	**130**	**127**	**121**	**142**	**152**	**142**	**165**	**169**	**164**
84				**18**	**7**	**9**	**64**	**43**	**40**	**140**	**85**	**96**	**152**	**114**	**117**	**150**	**117**	**110**	**152**	**151**	**143**	**170**	**179**	**168**	**175**	**181**	**190**
87				**19**	**8**	**9**	**70**	**48**	**43**	**167**	**100**	**113**	**182**	**137**	**139**	**177**	**136**	**128**	**185**	**182**	**170**	**203**	**214**	**202**	**211**	**217**	**223**
90				**18**	**8**	**9**	**74**	**49**	**46**	**192**		**175**	**213**	**160**	**162**	**210**	**159**	**151**	**217**	**213**	**205**	**244**	**255**	**238**	**250**	**254**	**249**

93				**19**	**8**	**9**	**78**	**52**	**48**	**225**		**197**	**249**	**187**	**189**	**245**	**185**	**175**	**256**	**255**	**240**	**289**	**303**	**288**	**305**	**315**	**307**
96				**20**	**8**	**9**	**82**	**56**	**50**	**261**		**220**	**294**	**219**	**222**	**291**	**217**	**206**	**302**	**303**	**285**	**345**	**360**	**345**	**345**	**339**	**345**
99				**21**	**8**	**10**	**86**	**57**	**51**	**295**		**245**	**338**	**256**	**259**	**337**	**247**	**240**	**353**	**356**	**334**	**396**	**417**	**400**	**437**	**429**	**437**
102				**21**	**8**	**10**	**87**	**59**	**52**	**329**		**267**	**383**	**290**	**295**	**388**	**283**	**278**	**404**	**401**	**389**	**433**	**440**	**447**	**434**	**425**	**432**
105				**21**	**8**	**10**	**90**	**60**	**54**	**364**		**291**	**419**	**332**	**337**	**421**	**325**	**318**	**434**	**428**	**436**	**436**	**443**	**453**	**432**	**422**	**431**
108				**21**	**8**	**10**	**92**	**61**	**55**	**395**		**313**	**436**	**368**	**384**	**429**	**352**	**364**	**444**	**430**	**449**	**431**	**438**	**449**	**429**	**421**	**428**
111				**22**	**8**	**10**	**94**	**61**	**54**	**414**		**333**	**436**	**396**	**424**	**425**	**364**	**400**	**440**	**427**	**447**	**430**	**435**	**442**	**425**	**417**	**423**
114				**22**	**8**	**10**	**96**	**63**	**55**	**425**		**354**	**432**	**412**	**460**	**422**	**374**	**432**	**438**	**423**	**446**	**424**	**431**	**445**	**426**	**414**	**421**
117				**23**	**8**	**10**	**96**	**63**	**55**	**431**		**370**	**431**	**434**	**475**	**423**	**391**	**451**	**435**	**423**	**448**	**423**	**429**	**442**	**425**	**416**	**421**
120				**23**	**8**	**10**	**97**	**63**	**55**	**432**		**385**	**428**	**454**	**476**	**417**	**409**	**447**	**434**	**417**	**441**	**421**	**426**	**437**	**422**	**410**	**418**
123				**24**	**8**	**11**	**99**	**63**	**56**	**435**		**394**	**428**	**459**	**471**	**417**	**422**	**435**	**430**	**413**	**439**	**419**	**424**	**433**	**420**	**410**	**415**
126				**24**	**8**	**10**	**100**	**63**	**56**	**432**		**400**	**428**	**450**	**461**	**412**	**424**	**422**	**426**	**408**	**438**	**417**	**420**	**430**	**417**	**408**	**413**
129				**24**	**8**	**11**	**101**	**64**	**56**	**432**		**405**	**431**	**446**	**460**	**413**	**422**	**418**	**427**	**411**	**437**	**414**	**423**	**435**	**414**	**403**	**410**
132				**25**	**8**	**11**	**103**	**65**	**57**	**433**		**405**	**425**	**444**	**456**	**412**	**415**	**409**	**425**	**407**	**436**	**411**	**415**	**427**	**413**	**400**	**408**
135				**25**	**8**	**11**	**103**	**64**	**56**	**433**		**407**	**428**	**438**	**451**	**409**	**409**	**408**	**421**	**406**	**430**	**410**	**415**	**426**	**413**	**402**	**410**
138				**25**	**8**	**11**	**104**	**65**	**56**	**429**		**405**	**425**	**437**	**448**	**406**	**407**	**402**	**423**	**404**	**431**	**409**	**414**	**427**	**413**	**402**	**408**
141				**26**	**8**	**11**	**106**	**64**	**57**	**427**		**403**	**425**	**432**	**445**	**405**	**399**	**397**	**418**	**403**	**427**	**407**	**414**	**425**	**407**	**397**	**402**
144				**26**	**8**	**11**	**106**	**66**	**56**	**428**		**404**	**424**	**431**	**442**	**402**	**398**	**394**	**418**	**403**	**426**	**407**	**413**	**422**	**411**	**400**	**403**
147				**26**	**8**	**12**	**107**	**65**	**56**	**424**		**402**	**423**	**427**	**440**	**404**	**392**	**391**	**417**	**399**	**429**	**407**	**414**	**421**	**408**	**396**	**404**

2.5x10^7 cfu/ml (60 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
60				**3**	**3**	**4**	**4**	**4**	**4**	**5**	**5**	**4**	**5**	**5**	**5**	**4**	**5**	**5**	**5**	**4**	**4**	**4**	**4**	**4**	**5**	**5**	**5**
63				**3**	**3**	**3**	**7**	**7**	**7**	**9**	**9**	**9**	**10**	**10**	**9**	**9**	**9**	**9**	**9**	**9**	**9**	**11**	**10**	**10**	**16**	**15**	**13**
66				**3**	**3**	**3**	**11**	**10**	**11**	**15**	**15**	**15**	**16**	**15**	**16**	**15**	**14**	**16**	**16**	**15**	**15**	**19**	**18**	**17**	**31**	**29**	**26**
69				**3**	**3**	**4**	**15**	**13**	**15**	**26**	**22**	**25**	**26**	**23**	**24**	**24**	**22**	**25**	**25**	**24**	**24**	**30**	**29**	**29**	**30**	**29**	**26**
72				**3**	**3**	**4**	**20**	**18**	**20**	**38**	**34**	**37**	**36**	**32**	**34**	**32**	**30**	**35**	**34**	**33**	**34**	**41**	**44**	**40**	**46**	**44**	**40**

75				**3**	**3**	**3**	**24**	**22**	**24**	**56**	**51**	**49**	**52**	**45**	**48**	**44**	**42**	**49**	**47**	**46**	**44**	**54**	**56**	**53**	**61**	**60**	**55**
78				**4**	**3**	**4**	**29**	**26**	**28**	**73**	**72**	**67**	**69**	**64**	**63**	**58**	**56**	**64**	**61**	**61**	**59**	**68**	**70**	**66**	**79**	**81**	**74**
81				**4**	**4**	**4**	**32**	**30**	**32**	**93**	**91**	**82**	**90**	**83**	**83**	**74**	**72**	**79**	**76**	**78**	**75**	**83**	**85**	**79**	**103**	**102**	**94**
84				**4**	**3**	**4**	**35**	**33**	**35**	**110**	**106**	**99**	**114**	**107**	**105**	**91**	**91**	**102**	**95**	**96**	**91**	**101**	**102**	**98**	**127**	**128**	**121**
87				**4**	**4**	**4**	**40**	**36**	**40**	**134**	**124**	**118**	**137**		**126**	**111**	**117**	**110**	**114**	**116**	**111**	**123**	**125**	**117**	**157**	**159**	**149**
90				**4**	**4**	**4**	**42**	**38**	**42**	**156**	**145**	**140**	**167**	**159**	**154**	**129**	**136**	**128**	**138**	**140**	**133**	**147**	**148**	**143**	**188**	**195**	**180**
93				**4**	**4**	**4**	**44**	**40**	**46**	**181**	**167**	**162**	**197**	**187**	**180**	**150**	**159**	**151**	**163**	**166**	**161**	**174**	**177**	**169**	**221**	**229**	**214**
96				**5**	**5**	**5**	**47**	**42**	**48**	**210**	**190**	**189**	**232**	**219**	**213**	**177**	**185**	**175**	**192**	**196**	**187**	**209**	**209**	**201**	**258**	**269**	**250**
99				**5**	**5**	**5**	**49**	**43**	**50**	**237**	**216**	**210**	**270**	**260**	**247**	**210**	**217**	**206**	**223**	**228**	**224**	**241**	**243**	**234**	**275**	**297**	**270**
102				**5**	**5**	**5**	**49**	**44**	**52**	**261**	**237**	**234**	**311**	**297**	**286**	**245**	**247**	**240**	**261**	**265**	**261**	**282**	**278**	**275**	**300**	**324**	**296**
105				**5**	**5**	**5**	**51**	**45**	**53**	**289**	**261**	**256**	**358**	**341**	**325**	**291**	**283**	**278**	**300**	**303**	**297**	**319**	**308**	**307**	**323**	**344**	**320**
108				**5**	**5**	**5**	**52**	**46**	**54**	**313**	**284**	**276**	**401**	**386**	**366**	**337**	**325**	**318**	**318**	**318**	**317**	**375**	**355**	**356**	**352**	**369**	**351**
111				**5**	**5**	**6**	**52**	**45**	**54**	**334**	**302**	**295**	**441**	**423**	**387**	**388**	**352**	**364**	**338**	**333**	**357**	**396**	**377**	**381**	**374**	**393**	**379**
114				**5**	**5**	**6**	**53**	**47**	**55**	**357**	**321**	**310**	**466**	**447**	**397**	**421**	**364**	**400**	**357**	**350**	**376**	**421**	**402**	**413**	**389**	**404**	**398**
117				**5**	**5**	**6**	**52**	**45**	**55**	**373**	**339**	**323**	**479**	**465**	**407**	**429**	**374**	**432**	**381**	**375**	**400**	**437**	**418**	**436**	**398**	**405**	**399**
120				**6**	**5**	**6**	**52**	**46**	**55**	**387**	**348**	**331**	**478**	**475**	**422**	**425**	**391**	**451**	**403**	**399**	**416**	**444**	**432**	**445**	**395**	**406**	**391**
123				**6**	**5**	**6**	**52**	**47**	**55**	**400**	**360**	**340**	**464**	**473**	**430**	**422**	**409**	**447**	**420**	**411**	**424**	**445**	**439**	**447**	**391**	**404**	**381**
126				**6**	**5**	**6**	**52**	**46**	**55**	**408**	**366**	**346**	**457**	**472**	**440**	**423**	**422**	**435**	**430**	**419**	**427**	**442**	**436**	**440**	**386**	**396**	**373**
129				**6**	**6**	**6**	**52**	**46**	**55**	**413**	**370**	**346**	**454**	**465**	**440**	**417**	**424**	**422**	**426**	**418**	**429**	**440**	**435**	**432**	**398**	**407**	**380**
132				**6**	**6**	**6**	**52**	**46**	**55**	**417**	**372**	**344**	**452**	**461**	**439**	**417**	**422**	**418**	**426**	**419**	**425**	**432**	**427**	**422**	**392**	**407**	**377**
135				**6**	**6**	**6**	**52**	**45**	**54**	**421**	**374**	**344**	**450**	**457**	**439**	**412**	**415**	**409**	**421**	**411**	**419**	**444**	**432**	**445**	**410**	**421**	**406**
138				**6**	**6**	**6**	**51**	**45**	**53**	**421**	**373**	**343**	**448**	**453**	**438**	**413**	**409**	**408**	**430**	**419**	**427**	**445**	**439**	**447**	**406**	**419**	**396**
141				**6**	**6**	**6**	**51**	**45**	**55**	**419**	**375**	**343**	**440**	**449**	**429**	**412**	**407**	**402**	**426**	**418**	**429**	**442**	**436**	**440**	**401**	**411**	**388**
144				**6**	**6**	**6**	**50**	**45**	**53**	**420**	**374**	**341**	**437**	**446**	**433**	**409**	**399**	**397**	**426**	**419**	**425**	**440**	**435**	**432**	**398**	**407**	**380**
147				**6**	**6**	**6**	**51**	**45**	**54**	**420**	**374**	**341**	**436**	**443**	**426**	**406**	**398**	**394**	**421**	**411**	**419**	**432**	**427**	**422**	**392**	**407**	**377**

10^7 cfu/ml (0 min)																										
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
3				2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6				2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	2	3
9				3	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
12				3	3	3	4	4	4	4	4	4	3	4	4	4	4	4	4	4	3	4	4	4	5	4	4
15				3	3	4	4	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	5	5
18				4	4	4	5	6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6
21				5	5	4	6	6	6	5	6	6	6	6	6	5	6	5	5	6	5	6	6	6	7	7	7
24				5	5	5	7	7	7	6	6	6	6	6	7	6	7	6	6	6	6	7	7	6	8	8	7
27				6	5	5	8	8	8	7	7	7	7	7	7	7	7	7	7	8	6	7	7	7	9	8	8
30				6	6	6	8	8	9	7	7	8	7		8	8	8	7	7	8	7	8	8	8	10	9	9
33				6	6	6	9	9	9	8	8	9	8	8	9	8	8	8	8	9	8	9	9	9	11	10	10
36				7	7	6	10	10	10	9	9	9	8	9	9	9	10	9	9	9	8	10	10	10	12	11	11
39				7	7	7	11	11	11	10	10	10	9	9	10	10	11	10	9	10	9	11	11	11	13	12	12
42				7	8	7	11	12	12	10	11	11	10	10	11	11	11	10	11	11	10	12	12	12	15	13	13
45				8	8	8	13	13	13	11	12	12	11	11	12	11	12	11	11	12	11	13	13	13	16	14	15
48				8	8	8	14	14	14	12	13	14	12	13	13	13	13	12	13	13	12	14	15	15	17	16	16
51				9	9	8	15	15	15	13	14	15	13	13	14	13	15	13	14	14	13	15	16	15	18	17	17
54				9	9	9	16	16	17	15	15	16	14	15	15	15	15	14	15	15	14	17	17	17	21	19	19
57				9	10	9	17	18	18	15	16	17	15	16	16	16	17	16	16	17	15	18	19	18	23	20	20
60				9	10	9	19	19	20	18	17	18	16	17	17	17	18	17	17	18	16	20	20	19	25	23	22
63				10	10	9	20	20	21	19	19	20	17	18	19	18	20	18	18	19	17	22	23	22	29	25	25
66				10	10	10	22	22	23	21	21	22	18	20	22	20	21	20	20	21	19	24	25	24	33	29	29
69				10	10	10	24	24	25	24	24	24	21	21	25	22	23	23	22	24	22	27	28	27	40	34	34
72				10	11	10	25	26	27	26	27	27	23	24	28	25	25	26	26	29	24	30	32	32	47	41	40
75				11	11	10	28	29	30	31	31	32	27	26	32	29	29	29	31	34	28	37	39	37	58	49	49
78				11	11	11	30	32	33	36	36	36	34	30	38	35	36	34	35	39	32	42	46	44	68	59	59
81				11	12	11	34	36	37	40	41	42	40	37	44	40	44	38	40	46	36	50	55	51	79	67	67

84				**11**	**11**	**11**	**37**	**38**	**40**	**47**	**47**	**49**	**47**	**45**	**50**	**48**	**53**	**44**	**48**	**54**	**43**	**58**	**63**	**60**	**91**	**80**	**78**
87				**11**	**12**	**11**	**40**	**41**	**43**	**54**	**56**	**58**	**54**	**53**	**58**	**55**	**63**	**52**	**56**	**63**	**50**	**68**	**74**	**71**	**106**	**92**	**90**
90				**12**	**12**	**11**	**42**	**44**	**46**	**61**	**64**	**64**	**62**	**64**	**67**	**64**	**72**	**61**	**65**	**73**	**58**	**79**	**85**	**82**	**123**	**105**	**103**
93				**12**	**12**	**11**	**45**	**46**	**49**	**71**	**72**	**74**	**74**	**75**	**77**	**74**	**85**	**70**	**75**	**84**	**67**	**91**	**98**	**94**	**141**	**121**	**119**
96				**12**	**12**	**11**	**48**	**49**	**51**	**79**	**81**	**82**	**83**	**85**	**87**	**84**	**96**	**80**	**85**	**96**	**77**	**106**	**114**	**108**	**159**	**138**	**134**
99				**12**	**12**	**11**	**49**	**50**	**51**	**89**	**89**	**90**	**96**	**101**	**101**	**96**	**110**	**90**	**99**	**111**	**88**	**120**	**130**	**125**	**180**	**157**	**153**
102				**12**	**12**	**11**	**51**	**53**	**52**	**99**	**100**	**100**	**111**	**116**	**117**	**108**	**126**	**105**	**113**	**129**	**102**	**138**	**147**	**145**	**203**	**178**	**170**
105				**12**	**12**	**11**	**52**	**53**	**54**	**107**	**108**	**107**	**123**	**129**	**130**	**125**	**144**	**118**	**128**	**146**	**115**	**155**	**164**	**162**	**227**	**199**	**190**
108				**12**	**13**	**11**	**53**	**54**	**55**	**116**	**116**	**115**	**138**	**147**	**148**	**141**	**162**	**135**	**144**	**164**	**131**	**174**	**186**	**181**	**254**	**222**	**216**
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114				**12**	**12**	**11**	**53**	**55**	**55**	**132**	**131**	**135**	**167**	**181**	**178**	**174**	**202**	**168**	**180**	**203**	**164**	**217**	**231**	**227**	**315**	**276**	**268**
117				**12**	**13**	**12**	**53**	**54**	**55**	**137**	**139**	**143**	**185**	**196**	**195**	**197**	**227**	**188**	**202**	**230**	**184**	**241**	**257**	**252**	**349**	**307**	**296**
120				**12**	**13**	**12**	**54**	**56**	**56**	**145**	**145**	**147**	**199**	**214**	**212**	**215**	**250**	**208**	**226**	**253**	**201**	**268**	**282**	**278**	**383**	**338**	**326**
123				**13**	**13**	**11**	**54**	**55**	**55**	**149**	**151**	**151**	**215**	**233**	**229**	**239**	**278**	**228**	**247**	**277**	**224**	**294**	**314**	**308**	**422**	**375**	**363**
126				**12**	**13**	**12**	**55**	**55**	**56**	**156**	**154**	**152**	**232**	**251**	**247**	**263**	**305**	**252**	**272**	**307**	**249**	**327**	**346**	**340**	**441**	**413**	**401**
129				**12**	**13**	**11**	**55**	**56**	**56**	**159**	**159**	**152**	**246**	**268**	**262**	**290**	**335**	**277**	**303**	**338**	**273**	**361**	**378**	**372**	**443**	**443**	**432**
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141				**13**	**13**	**12**	**56**	**56**	**57**	**164**	**164**	**159**	**300**	**328**	**328**	**410**	**454**	**392**	**430**	**457**	**395**	**465**	**453**	**463**	**424**	**437**	**433**
144				**12**	**13**	**12**	**55**	**56**	**56**	**165**	**163**	**159**	**306**	**336**	**339**	**441**	**463**	**425**	**450**	**457**	**425**	**464**	**447**	**465**	**417**	**433**	**433**
147				**13**	**13**	**12**	**55**	**57**	**56**	**166**	**163**	**159**	**318**	**346**	**349**	**465**	**463**	**448**	**455**	**456**	**444**	**461**	**441**	**464**	**416**	**428**	**431**
150				**13**	**13**	**12**	**55**	**56**	**56**	**168**	**165**	**160**	**326**	**358**	**359**	**476**	**458**	**464**	**456**	**446**	**452**	**460**	**438**	**462**	**410**	**426**	**426**
153				**13**	**13**	**11**	**57**	**58**	**56**	**168**	**164**	**160**	**331**	**359**	**367**	**475**	**454**	**463**	**450**	**444**	**446**	**457**	**433**	**457**	**407**	**424**	**424**
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159				**13**	**13**	**12**	**56**	**57**	**57**	**169**	**164**	**160**	**338**	**367**	**373**	**470**	**443**	**457**	**443**	**435**	**446**	**452**	**421**	**455**	**399**	**416**	**418**
162				**13**	**13**	**12**	**56**	**56**	**56**	**167**	**166**	**160**	**340**	**371**	**377**	**464**	**439**	**452**	**441**	**434**	**446**	**449**	**416**	**453**	**393**	**411**	**414**
165				**13**	**13**	**12**	**56**	**57**	**57**	**171**	**166**	**161**	**341**	**371**	**378**	**461**	**436**	**448**	**436**	**430**	**441**	**441**	**412**	**449**	**389**	**408**	**411**

10^7 cfu/ml (30 min)																											
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
30				3	3	3	3	3	3	3	4	3	3	3	3	3	4	3	4	3	4	4	3	4	3	3	3
33				4	4	3	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4
36				4	4	4	6	5	5	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	5	5	5
39				5	5	5	6	7	6	7	7	7	8	7	7	7	7	7	7	7	7	7	7	7	7	6	6
42				6	5	5	8	8	7	8	8	8	8	8	8	8	9	8	9	9	9	8	8	9	8	8	7
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51				7	7	6	11	12	10	12	12	12	12	12	12	12	12	12	13	12	12	13	12	14	12	12	11
54				8	7	7	11	12	11	13	13	14	14	14	14	13	14	14	14	14	14	14	14	16	14	13	13
57				8	8	7	12	14	12	14	14	15	15		15	15	16	15	15	15	15	15	15	18	14	14	14
60				9	8	7	13	14	13	16	15	16	16	17	17	16	17	16	16	16	16	17	17	19	16	15	15
63				9	8	8	14	15	14	17	17	17	17	18	18	17	18	17	18	17	17	17	18	20	17	16	16
66				10	8	8	14	16	14	18	18	19	19	20	20	19	20	19	19	19	18	19	20	22	18	18	18
69				10	9	8	15	17	15	20	19	20	20	21	21	20	22	21	20	21	20	20	21	24	20	20	20
72				11	9	9	16	17	16	22	21	22	22	23	23	22	24	22	22	22	21	22	23	26	22	21	21
75				11	10	9	16	19	16	23	22	23	24	25	24	24	25	24	24	24	23	24	25	27	25	24	23
78				12	10	10	17	19	17	25	23	26	26	27	26	25	28	26	25	26	24	26	27	30	27	26	26
81				12	11	10	18	21	18	26	25	27	27	29	29	28	30	28	27	28	27	29	30	32	31	30	29
84				13	11	10	19	22	19	29	26	29	30	31	31	31	33	30	30	31	29	32	33	36	35	34	33
87				13	12	11	20	20	20	32	29	32	34	35	35	36	38	34	34	35	33	37	38	41	42	41	40
90				14	12	11	20	21	21	35	32	35	38	40	40	40	44	39	38	39	36	42	43	47	51	49	48
93				16	15	14	21	21	21	39	35	39	44	45	44	45	51	45	44	44	41	50	51	53	62	60	58
96				16	15	14	21	22	21	43	40	44	51	52	51	52	59	52	51	51	48	61	61	63	76	74	74
99				16	15	14	22	23	22	49	45	50	60	59	57	62	69	59	62	60	55	74	73	76	93	91	92
102				16	15	14	22	23	23	55	51	55	69	69	66	72	80	69	73	70	66	89	86	90	114	110	110
105				17	15	14	23	24	23	61	59	62	82	79	78	84	94	82	88	85	81	110	107	109	141	137	137

108				**16**	**15**	**14**	**24**	**24**	**24**	**69**	**65**	**68**	**96**	**95**	**92**	**99**	**108**	**96**	**105**	**100**	**96**	**130**	**128**	**132**	**172**	**165**	**166**
111				**17**	**15**	**15**	**24**	**25**	**23**	**76**	**72**	**75**	**114**	**110**	**107**	**117**	**128**	**113**	**124**	**119**	**114**	**157**	**153**	**156**	**207**	**201**	**203**
114				**17**	**16**	**15**	**24**	**25**	**24**	**85**	**80**	**83**	**133**	**129**	**123**	**140**	**152**	**136**	**151**	**144**	**139**	**190**	**183**	**189**	**251**	**246**	**245**
117				**18**	**16**	**15**	**25**	**26**	**25**	**93**	**86**	**90**	**156**	**148**	**143**	**168**	**177**	**161**	**181**	**169**	**170**	**236**	**222**	**237**	**300**	**292**	**292**
120				**18**	**16**	**15**	**25**	**26**	**25**	**102**	**94**	**98**	**178**	**169**	**161**	**197**	**215**	**189**	**217**	**205**	**205**	**276**	**265**	**274**	**348**	**343**	**341**
123				**18**	**16**	**15**	**25**	**26**	**25**	**108**	**99**	**105**	**204**	**188**	**184**	**231**	**251**	**222**	**259**	**242**	**243**	**325**	**315**	**321**	**391**	**390**	**392**
126				**19**	**16**	**15**	**26**	**27**	**26**	**113**	**106**	**110**	**230**	**211**	**205**	**265**	**286**	**258**	**299**	**282**	**287**	**381**	**362**	**378**	**402**	**405**	**417**
129				**19**	**17**	**16**	**26**	**27**	**26**	**117**	**110**	**116**	**250**	**234**	**224**	**307**	**327**	**298**	**353**	**329**	**341**	**444**	**430**	**441**	**399**	**403**	**419**
132				**19**	**17**	**16**	**26**	**28**	**26**	**122**	**115**	**121**	**269**	**252**	**244**	**344**	**368**	**336**	**413**	**382**	**393**	**489**	**479**	**487**	**397**	**402**	**412**
135				**20**	**17**	**16**	**27**	**27**	**27**	**126**	**118**	**125**	**286**	**272**	**262**	**390**	**420**	**381**	**469**	**441**	**455**	**500**	**496**	**507**	**396**	**399**	**408**
138				**21**	**17**	**16**	**27**	**28**	**27**	**130**	**121**	**129**	**307**	**291**	**278**	**432**	**456**	**425**	**502**	**474**	**489**	**494**	**493**	**502**	**392**	**397**	**409**
141				**21**	**18**	**16**	**27**	**28**	**27**	**132**	**124**	**132**	**323**	**304**	**293**	**467**	**488**	**458**	**508**	**480**	**497**	**494**	**492**	**502**	**390**	**394**	**405**
144				**21**	**18**	**17**	**28**	**28**	**27**	**135**	**127**	**135**	**333**	**315**	**303**	**476**	**495**	**478**	**503**	**478**	**492**	**488**	**487**	**499**	**388**	**393**	**405**
147				**22**	**18**	**17**	**28**	**29**	**28**	**138**	**130**	**137**	**342**	**324**	**314**	**480**	**496**	**487**	**499**	**469**	**489**	**487**	**484**	**494**	**386**	**391**	**402**
150				**22**	**18**	**17**	**28**	**29**	**28**	**140**	**131**	**140**	**351**	**330**	**323**	**475**	**492**	**488**	**494**	**466**	**485**	**484**	**479**	**493**	**385**	**389**	**400**
153				**20**	**17**	**16**	**28**	**29**	**28**	**143**	**133**	**143**	**357**	**338**	**330**	**477**	**491**	**485**	**492**	**466**	**483**	**481**	**478**	**492**	**384**	**391**	**397**
156				**21**	**17**	**16**	**29**	**29**	**29**	**146**	**136**	**146**	**360**	**344**	**338**	**478**	**491**	**489**	**494**	**465**	**488**	**485**	**480**	**495**	**381**	**387**	**397**
159				**21**	**18**	**16**	**29**	**30**	**29**	**148**	**139**	**148**	**366**	**348**	**345**	**472**	**489**	**486**	**493**	**459**	**486**	**482**	**478**	**492**	**379**	**386**	**398**
162				**21**	**18**	**17**	**29**	**30**	**29**	**149**	**139**	**150**	**369**	**354**	**348**	**471**	**492**	**487**	**492**	**461**	**483**	**478**	**476**	**488**	**381**	**386**	**396**
165				**22**	**18**	**17**	**30**	**31**	**29**	**152**	**141**	**153**	**372**	**357**	**349**	**473**	**488**	**485**	**496**	**463**	**485**	**484**	**479**	**489**	**380**	**386**	**393**
168				**22**	**18**	**17**	**30**	**31**	**30**	**154**	**143**	**155**	**374**	**360**	**351**	**468**	**484**	**483**	**491**	**461**	**481**	**480**	**477**	**489**	**380**	**383**	**394**
171				**19**	**16**	**15**	**30**	**31**	**30**	**156**	**145**	**157**	**377**	**364**	**355**	**470**	**485**	**483**	**489**	**457**	**480**	**477**	**474**	**491**	**381**	**385**	**393**
174				**19**	**17**	**16**	**31**	**32**	**30**	**159**	**147**	**160**	**379**	**365**	**357**	**471**	**489**	**483**	**495**	**459**	**482**	**479**	**476**	**491**	**378**	**383**	**390**
177				**19**	**17**	**16**	**32**	**32**	**31**	**160**	**149**	**161**	**380**	**368**	**360**	**465**	**484**	**481**	**491**	**456**	**481**	**476**	**474**	**486**	**376**	**384**	**390**
180				**20**	**17**	**16**	**31**	**32**	**31**	**162**	**150**	**163**	**384**	**372**	**362**	**465**	**483**	**480**	**493**	**458**	**481**	**475**	**473**	**486**	**377**	**382**	**392**
183				**21**	**17**	**16**	**32**	**32**	**31**	**164**	**153**	**167**	**384**	**373**	**363**	**467**	**481**	**481**	**496**	**459**	**485**	**479**	**477**	**487**	**374**	**382**	**387**
186				**21**	**18**	**16**	**32**	**33**	**32**	**164**	**153**	**169**	**386**	**374**	**366**	**461**	**481**	**477**	**491**	**452**	**480**	**478**	**472**	**482**	**375**	**381**	**388**
189				**21**	**18**	**17**	**33**	**34**	**32**	**165**	**157**	**171**	**387**	**377**	**367**	**462**	**481**	**480**	**489**	**455**	**480**	**477**	**472**	**481**	**374**	**381**	**388**
192				**22**	**18**	**17**	**33**	**34**	**32**	**168**	**157**	**172**	**389**	**379**	**367**	**460**	**480**	**481**	**489**	**457**	**478**	**479**	**472**	**482**	**370**	**378**	**384**

195				**22**	**18**	**17**	**34**	**35**	**33**	**171**	**158**	**174**	**391**	**381**	**369**	**458**	**476**	**475**	**489**	**453**	**478**	**473**	**471**	**480**	**370**	**376**	**385**
198				**20**	**17**	**16**	**34**	**36**	**33**	**171**	**159**	**177**	**393**	**384**	**373**	**459**	**476**	**478**	**487**	**453**	**477**	**472**	**471**	**478**	**370**	**377**	**381**
201				**21**	**17**	**16**	**35**	**35**	**34**	**173**	**162**	**179**	**395**	**390**	**378**	**460**	**479**	**479**	**488**	**449**	**475**	**475**	**471**	**482**	**367**	**372**	**381**
204				**21**	**18**	**16**	**35**	**36**	**35**	**175**	**163**	**180**	**396**	**387**	**375**	**456**	**473**	**477**	**483**	**447**	**472**	**472**	**468**	**478**	**366**	**374**	**379**
207				**21**	**18**	**17**	**36**	**37**	**35**	**177**	**164**	**182**	**395**	**387**	**373**	**452**	**472**	**472**	**482**	**443**	**474**	**470**	**467**	**478**	**367**	**372**	**379**
210				**22**	**18**	**17**	**36**	**37**	**35**	**177**	**166**	**184**	**399**	**392**	**377**	**455**	**472**	**473**	**485**	**445**	**471**	**473**	**471**	**480**	**366**	**372**	**377**
213				**22**	**18**	**17**	**38**	**38**	**35**	**179**	**166**	**184**	**395**	**388**	**377**	**453**	**469**	**472**	**478**	**443**	**474**	**474**	**469**	**479**	**366**	**370**	**379**
216				**22**	**18**	**17**	**38**	**38**	**36**	**180**	**167**	**187**	**395**	**387**	**378**	**450**	**474**	**472**	**479**	**443**	**471**	**472**	**470**	**479**	**366**	**371**	**376**
219				**22**	**18**	**17**	**38**	**38**	**37**	**183**	**168**	**188**	**400**	**394**	**382**	**453**	**472**	**473**	**480**	**444**	**471**	**475**	**471**	**482**	**366**	**370**	**376**
222				**20**	**17**	**16**	**40**	**39**	**37**	**184**	**170**	**191**	**400**	**395**	**380**	**449**	**467**	**472**	**480**	**443**	**468**	**470**	**470**	**478**	**362**	**368**	**374**
225				**21**	**17**	**16**	**40**	**40**	**38**	**186**	**173**	**193**	**401**	**394**	**382**	**450**	**467**	**471**	**477**	**441**	**467**	**471**	**467**	**473**	**362**	**367**	**372**
228				**21**	**18**	**16**	**41**	**40**	**38**	**187**	**173**	**194**	**402**	**394**	**385**	**449**	**468**	**471**	**478**	**442**	**469**	**474**	**468**	**477**	**360**	**365**	**372**
231				**21**	**18**	**17**	**42**	**41**	**39**	**188**	**176**	**196**	**402**	**393**	**383**	**446**	**464**	**472**	**476**	**439**	**468**	**474**	**464**	**476**	**360**	**365**	**369**

10^7 cfu/ml (60 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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120				**11**	**14**	**14**	**27**	**24**	**23**	**74**	**90**	**78**	**117**	**118**	**103**	**120**	**123**	**134**	**132**	**132**	**134**	**138**	**132**	**172**	**202**	**221**	**245**
123				**11**	**15**	**15**	**28**	**25**	**24**	**79**	**97**	**86**	**132**	**134**	**120**	**142**	**145**	**158**	**157**	**159**	**163**	**171**	**162**	**212**	**238**	**262**	**297**
126				**12**	**16**	**16**	**29**	**26**	**25**	**85**	**101**	**91**	**145**	**148**	**131**	**166**	**172**	**183**	**185**	**187**	**193**	**201**	**189**	**249**	**272**	**307**	**346**
129				**12**	**16**	**16**	**30**	**27**	**26**	**89**	**108**	**95**	**160**	**162**	**143**	**191**	**196**	**212**	**216**	**218**	**226**	**238**	**225**	**297**	**309**	**359**	**393**
132				**13**	**17**	**17**	**31**	**28**	**27**	**92**	**111**	**98**	**175**	**177**	**155**	**219**	**227**	**243**	**248**	**253**	**263**	**280**	**260**	**347**	**350**	**403**	**411**
135				**14**	**18**	**19**	**34**	**29**	**28**	**95**	**117**	**101**	**186**	**188**	**167**	**248**	**256**	**276**	**288**	**295**	**307**	**333**	**310**	**413**	**385**	**409**	**409**
138				**14**	**20**	**20**	**35**	**30**	**29**	**98**	**118**	**103**	**198**	**197**	**176**	**275**	**286**	**308**	**325**	**330**	**352**	**375**	**355**	**459**	**383**	**402**	**400**
141				**15**	**21**	**21**	**37**	**31**	**30**	**101**	**123**	**106**	**210**	**208**	**185**	**312**	**324**	**347**	**368**	**375**	**406**	**427**	**396**	**485**	**373**	**398**	**396**
144				**16**	**23**	**23**	**38**	**32**	**31**	**102**	**126**	**107**	**221**	**219**	**193**	**332**	**351**	**377**	**391**	**403**	**449**	**462**	**430**	**477**	**365**	**393**	**396**
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150				**18**	**26**	**27**	**41**	**34**	**33**	**105**	**129**	**113**	**238**	**233**	**208**	**383**	**407**	**440**	**432**	**446**	**465**	**457**	**436**	**467**	**356**	**384**	**390**
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180				**29**	**29**	**29**	**58**	**51**	**47**	**115**	**153**	**132**	**272**	**270**	**240**	**434**	**442**	**450**	**391**	**406**	**425**	**408**	**392**	**435**	**331**	**360**	**377**
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186				**29**	**30**	**29**	**60**	**53**	**50**	**117**	**157**	**135**	**275**	**275**	**246**	**429**	**441**	**447**	**388**	**402**	**426**	**404**	**384**	**435**	**331**	**361**	**377**
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201				**32**	**32**	**31**	**59**	**52**	**58**	**120**	**167**	**143**	**283**	**282**	**254**	**421**	**431**	**439**	**380**	**396**	**415**	**397**	**382**	**431**	**324**	**354**	**368**
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216				**33**	**34**	**32**	**59**	**53**	**53**	**125**	**178**	**151**	**293**	**294**	**256**	**413**	**426**	**431**	**377**	**391**	**406**	**393**	**377**	**427**	**323**	**352**	**365**
219				**33**	**34**	**32**	**60**	**53**	**54**	**124**	**179**	**152**	**288**	**288**	**258**	**411**	**421**	**426**	**374**	**387**	**407**	**393**	**372**	**426**	**322**	**352**	**366**
222				**34**	**35**	**33**	**59**	**53**	**55**	**125**	**180**	**154**	**293**	**295**	**258**	**409**	**422**	**426**	**373**	**387**	**407**	**392**	**373**	**427**	**325**	**351**	**364**
225				**34**	**36**	**33**	**59**	**52**	**57**	**126**	**182**	**155**	**295**	**295**	**259**	**410**	**424**	**428**	**374**	**389**	**406**	**393**	**373**	**425**	**325**	**351**	**366**
228				**35**	**35**	**34**	**59**	**52**	**58**	**126**	**186**	**158**	**297**	**297**	**262**	**411**	**420**	**424**	**373**	**390**	**406**	**390**	**372**	**423**	**322**	**350**	**364**
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5x10^6 cfu/ml (0 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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5x10^6 cfu/ml (30 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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99				**12**	**11**	**12**	**18**	**18**	**17**	**28**	**27**	**25**	**35**	**32**	**33**	**37**	**38**	**38**	**44**	**42**	**41**	**48**	**45**	**44**	**62**	**55**	**54**
102				**12**	**11**	**12**	**18**	**17**	**18**	**29**	**28**	**26**	**38**	**35**	**36**	**42**	**43**	**43**	**49**	**48**	**46**	**54**	**51**	**51**	**74**	**66**	**65**
105				**12**	**11**	**12**	**18**	**18**	**18**	**31**	**29**	**27**	**42**	**38**	**39**	**47**	**49**	**48**	**57**	**54**	**52**	**65**	**59**	**58**	**89**	**79**	**78**
108				**12**	**12**	**12**	**19**	**18**	**19**	**32**	**30**	**28**	**45**	**41**	**42**	**53**	**55**	**53**	**66**	**63**	**59**	**75**	**69**	**68**	**108**	**95**	**92**
111				**12**	**12**	**12**	**19**	**18**	**19**	**32**	**31**	**29**	**48**	**44**	**45**	**58**	**62**	**60**	**77**	**72**	**69**	**89**	**82**	**81**	**128**	**114**	**111**
114				**12**	**12**	**12**	**19**	**19**	**19**	**34**	**33**	**30**	**51**	**47**	**48**	**67**	**70**	**68**	**89**	**86**	**82**	**107**	**98**	**96**	**156**	**136**	**134**
117				**13**	**12**	**12**	**20**	**19**	**20**	**35**	**34**	**32**	**55**	**49**	**51**	**75**	**78**	**79**	**106**	**98**	**96**	**132**	**116**	**115**	**188**	**170**	**164**
120				**13**	**12**	**12**	**20**	**19**	**20**	**36**	**35**	**33**	**58**	**53**	**54**	**85**	**87**	**85**	**126**	**118**	**112**	**154**	**139**	**137**	**225**	**203**	**192**
123				**13**	**13**	**13**	**20**	**19**	**20**	**37**	**35**	**33**	**61**	**54**	**57**	**91**	**95**	**95**	**143**	**134**	**129**	**177**	**162**	**160**	**268**	**240**	**231**
126				**13**	**12**	**13**	**20**	**20**	**20**	**37**	**37**	**34**	**64**	**56**	**60**	**100**	**103**	**104**	**159**	**150**	**147**	**203**	**188**	**186**	**316**	**284**	**277**
129				**13**	**13**	**13**	**20**	**20**	**21**	**39**	**37**	**35**	**67**	**60**	**62**	**110**	**115**	**117**	**180**	**170**	**166**	**240**	**220**	**219**	**372**	**335**	**324**
132				**13**	**13**	**13**	**20**	**20**	**20**	**39**	**38**	**35**	**69**	**62**	**65**	**118**	**123**	**127**	**204**	**190**	**185**	**277**	**255**	**252**	**424**	**386**	**371**
135				**13**	**13**	**13**	**21**	**20**	**21**	**39**	**39**	**35**	**72**	**64**	**67**	**124**	**134**	**140**	**226**	**215**	**205**	**319**	**298**	**296**	**472**	**443**	**431**
138				**13**	**13**	**14**	**21**	**21**	**21**	**39**	**39**	**36**	**72**	**66**	**69**	**133**	**144**	**149**	**247**	**237**	**222**	**352**	**335**	**333**	**483**	**470**	**484**
141				**14**	**13**	**14**	**21**	**21**	**21**	**41**	**40**	**37**	**74**	**67**	**70**	**140**	**151**	**158**	**265**	**253**	**241**	**394**	**373**	**371**	**482**	**471**	**493**
144				**14**	**13**	**14**	**21**	**21**	**22**	**41**	**40**	**37**	**75**	**67**	**72**	**145**	**159**	**161**	**281**	**274**	**260**	**433**	**415**	**410**	**479**	**468**	**493**
147				**14**	**13**	**14**	**22**	**21**	**22**	**42**	**40**	**37**	**77**	**68**	**72**	**151**	**163**	**167**	**296**	**293**	**282**	**466**	**455**	**454**	**476**	**467**	**483**

150				**14**	**13**	**13**	**21**	**21**	**22**	**41**	**41**	**38**	**77**	**70**	**72**	**155**	**169**	**173**	**306**	**307**	**291**	**486**	**481**	**484**	**473**	**464**	**483**
153				**14**	**13**	**14**	**22**	**21**	**22**	**42**	**41**	**38**	**77**	**70**	**74**	**159**	**173**	**178**	**313**	**319**	**305**	**503**	**495**	**506**	**474**	**462**	**479**
156				**14**	**13**	**14**	**22**	**21**	**22**	**42**	**41**	**39**	**78**	**70**	**75**	**162**	**178**	**180**	**326**	**328**	**319**	**510**	**505**	**520**	**472**	**461**	**479**
159				**14**	**13**	**14**	**22**	**22**	**22**	**42**	**41**	**39**	**78**	**71**	**74**	**164**	**182**	**183**	**331**	**336**	**328**	**505**	**503**	**522**	**469**	**457**	**478**
162				**14**	**14**	**14**	**22**	**21**	**22**	**43**	**42**	**39**	**79**	**71**	**75**	**166**	**183**	**185**	**341**	**344**	**336**	**503**	**501**	**519**	**465**	**453**	**476**
165				**14**	**14**	**14**	**22**	**22**	**22**	**43**	**42**	**40**	**79**	**72**	**75**	**167**	**186**	**187**	**345**	**349**	**341**	**505**	**500**	**519**	**464**	**455**	**470**
168				**15**	**14**	**14**	**22**	**22**	**23**	**43**	**42**	**40**	**78**	**73**	**76**	**169**	**185**	**189**	**348**	**356**	**347**	**500**	**500**	**516**	**457**	**450**	**466**
171				**14**	**14**	**14**	**22**	**22**	**23**	**44**	**42**	**40**	**80**	**72**	**76**	**170**	**188**	**191**	**355**	**361**	**352**	**500**	**498**	**513**	**457**	**449**	**459**
174				**15**	**14**	**14**	**23**	**22**	**23**	**44**	**43**	**40**	**80**	**72**	**77**	**170**	**188**	**190**	**355**	**362**	**351**	**499**	**500**	**515**	**455**	**450**	**460**
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201				**15**	**14**	**15**	**24**	**23**	**24**	**45**	**44**	**41**	**82**	**75**	**78**	**175**	**194**	**198**	**370**	**381**	**373**	**487**	**486**	**506**	**432**	**426**	**431**
204				**15**	**15**	**15**	**24**	**23**	**24**	**45**	**44**	**42**	**81**	**75**	**78**	**176**	**196**	**198**	**370**	**381**	**371**	**485**	**488**	**505**	**429**	**425**	**425**
207				**16**	**15**	**15**	**24**	**24**	**24**	**45**	**45**	**41**	**81**	**74**	**78**	**175**	**195**	**197**	**372**	**381**	**372**	**487**	**487**	**503**	**428**	**419**	**425**
210				**16**	**15**	**15**	**24**	**24**	**24**	**45**	**45**	**42**	**82**	**75**	**79**	**175**	**195**	**197**	**372**	**380**	**372**	**484**	**488**	**503**	**425**	**420**	**419**
213				**16**	**15**	**16**	**24**	**23**	**24**	**45**	**45**	**42**	**82**	**75**	**79**	**176**	**195**	**199**	**371**	**381**	**372**	**485**	**484**	**502**	**423**	**414**	**420**
216				**16**	**15**	**15**	**24**	**24**	**24**	**45**	**45**	**41**	**82**	**74**	**78**	**176**	**197**	**198**	**372**	**382**	**374**	**482**	**483**	**501**	**422**	**414**	**419**
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222				**16**	**15**	**16**	**24**	**24**	**24**	**45**	**45**	**42**	**82**	**75**	**78**	**179**	**197**	**199**	**373**	**381**	**374**	**484**	**483**	**501**	**418**	**411**	**413**
225				**16**	**15**	**16**	**25**	**24**	**24**	**46**	**45**	**42**	**83**	**76**	**79**	**176**	**197**	**200**	**373**	**384**	**373**	**479**	**480**	**498**	**414**	**409**	**412**
228				**16**	**15**	**16**	**25**	**25**	**25**	**46**	**46**	**42**	**83**	**75**	**80**	**176**	**198**	**200**	**374**	**383**	**374**	**480**	**482**	**498**	**415**	**405**	**412**
231				**16**	**16**	**16**	**25**	**24**	**25**	**46**	**45**	**42**	**84**	**76**	**79**	**177**	**198**	**200**	**374**	**383**	**377**	**477**	**480**	**496**	**411**	**402**	**406**

5x10^6 cfu/ml (60 min)																										
time (min)	5x10^8 pfu/ml			5x10^7 pfu/ml			5x10^6 pfu/ml			5x10^5 pfu/ml			5x10^4 pfu/ml			5x10^3 pfu/ml			5x10^2 pfu/ml			5x10^1 pfu/ml			0 pfu/ml		
60				3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3
63				3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	5	4
66				3	3	3	4	4	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	6	6	6
69				4	4	4	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6	7	7	7	8	8	7
72				4	4	4	6	6	5	7	7	7	7	7	7	7	7	7	8	7	8	8	8	8	9	9	9
75				4	4	4	6	6	6	8	8	8	9	8	9	9	8	9	9	9	9	10	10	10	11	11	11
78				5	5	5	7	7	7	10	9	9	10	10	10	10	10	10	11	10	11	12	11	11	13	13	13
81				5	5	5	7	8	8	11	11	11	12	11	12	12	12	12	12	13	13	13	14	13	16	16	16
84				5	5	5	8	8	8	13	12	13	14	13	14	14	14	14	15	14	14	15	16	15	18	19	19
87				5	5	5	9	9	9	14	14	14	15		15	16	15	15	16	16	17	18	18	17	21	21	22
90				5	5	5	9	9	9	15	15	16	17	17	18	18	17	18	19	19	19	20	20	20	23	24	25
93				6	6	6	10	10	10	17	17	17	20	19	20	21	20	20	21	22	22	24	23	23	27	28	29
96				6	6	6	10	10	10	19	18	19	22	21	22	23	22	23	24	24	24	27	26	26	31	32	33
99				6	6	6	11	11	11	20	20	21	25	24	25	27	26	26	26	26	27	29	29	29	35	36	38
102				6	6	6	11	11	11	22	22	22	27	27	28	31	29	29	30	31	32	34	33	34	41	42	45
105				7	7	7	12	12	12	23	23	23	30	29	30	35	33	33	35	35	36	39	39	38	49	50	54
108				7	7	7	12	12	12	25	25	25	33	32	33	40	37	38	40	40	42	45	44	44	59	60	66
111				7	7	7	13	13	12	26	26	26	37	36	37	45	43	43	46	47	49	53	53	53	72	74	80
114				7	7	7	13	13	13	27	26	28	40	39	40	52	48	49	54	53	57	63	63	63	88	90	97
117				7	8	7	13	13	13	28	28	29	44	42	43	58	54	55	64	62	68	76	75	76	105	108	118
120				8	8	7	14	14	13	30	29	30	47	45	47	65	62	64	77	76	81	91	91	90	129	132	144
123				8	8	8	14	14	14	32	30	32	51	49	50	76	69	72	91	91	97	111	110	110	158	163	177
126				8	8	8	14	14	14	32	31	32	53	51	53	83	77	78	108	104	112	130	130	130	192	196	216
129				8	8	8	14	15	14	33	32	33	56	55	55	92	85	87	122	120	128	153	154	154	228	233	254
132				8	8	8	14	15	14	34	33	34	59	58	58	101	94	96	140	137	146	182	183	181	273	282	305
135				8	8	8	15	15	14	35	34	35	63	61	62	114	104	106	159	157	169	211	213	212	324	335	361

138				**8**	**9**	**8**	**15**	**15**	**15**	**36**	**35**	**35**	**65**	**63**	**65**	**125**	**111**	**115**	**177**	**173**	**190**	**248**	**251**	**244**	**378**	**388**	**415**
141				**8**	**9**	**8**	**15**	**16**	**15**	**36**	**36**	**37**	**67**	**66**	**67**	**136**	**121**	**125**	**197**	**192**	**210**	**289**	**294**	**287**	**437**	**450**	**465**
144				**9**	**9**	**9**	**15**	**16**	**15**	**37**	**36**	**37**	**70**	**68**	**70**	**146**	**131**	**135**	**215**	**212**	**234**	**327**	**333**	**324**	**480**	**488**	**470**
147				**9**	**9**	**9**	**15**	**16**	**16**	**37**	**37**	**38**	**72**	**69**	**70**	**155**	**140**	**143**	**230**	**227**	**249**	**370**	**375**	**363**	**491**	**494**	**472**
150				**9**	**9**	**9**	**16**	**16**	**16**	**38**	**37**	**39**	**72**	**70**	**72**	**162**	**149**	**151**	**250**	**246**	**275**	**407**	**410**	**400**	**485**	**492**	**471**
153				**9**	**9**	**9**	**16**	**17**	**16**	**38**	**38**	**39**	**73**	**71**	**73**	**168**	**155**	**157**	**268**	**267**	**288**	**444**	**447**	**435**	**482**	**487**	**469**
156				**9**	**9**	**9**	**16**	**16**	**16**	**39**	**38**	**38**	**74**	**72**	**73**	**175**	**161**	**162**	**280**	**278**	**309**	**473**	**473**	**464**	**481**	**485**	**464**
159				**9**	**9**	**9**	**16**	**16**	**16**	**39**	**39**	**39**	**75**	**73**	**74**	**179**	**167**	**169**	**292**	**293**	**327**	**491**	**490**	**480**	**480**	**483**	**463**
162				**9**	**9**	**9**	**17**	**17**	**17**	**40**	**39**	**40**	**76**	**74**	**75**	**184**	**171**	**174**	**307**	**306**	**341**	**498**	**499**	**494**	**478**	**484**	**464**
165				**9**	**10**	**9**	**17**	**16**	**16**	**40**	**39**	**40**	**76**	**75**	**75**	**187**	**173**	**176**	**313**	**313**	**350**	**500**	**498**	**493**	**475**	**482**	**462**
168				**9**	**10**	**10**	**17**	**17**	**17**	**40**	**39**	**40**	**76**	**75**	**75**	**191**	**175**	**177**	**324**	**323**	**360**	**501**	**498**	**495**	**473**	**481**	**457**
171				**9**	**10**	**10**	**17**	**18**	**16**	**41**	**40**	**41**	**77**	**76**	**77**	**192**	**179**	**180**	**326**	**328**	**364**	**500**	**496**	**493**	**468**	**476**	**454**
174				**10**	**10**	**10**	**18**	**17**	**17**	**41**	**40**	**41**	**78**	**76**	**77**	**195**	**180**	**182**	**331**	**331**	**369**	**496**	**495**	**491**	**466**	**471**	**452**
177				**10**	**10**	**10**	**18**	**18**	**17**	**41**	**40**	**41**	**78**	**75**	**77**	**196**	**182**	**183**	**336**	**336**	**376**	**497**	**494**	**489**	**465**	**469**	**453**
180				**10**	**10**	**10**	**18**	**18**	**17**	**41**	**41**	**42**	**78**	**76**	**78**	**197**	**183**	**183**	**335**	**337**	**376**	**495**	**494**	**491**	**465**	**469**	**448**
183				**10**	**10**	**10**	**18**	**18**	**17**	**42**	**40**	**42**	**79**	**76**	**77**	**198**	**184**	**184**	**340**	**345**	**381**	**492**	**496**	**488**	**461**	**468**	**447**
186				**10**	**11**	**10**	**18**	**18**	**18**	**42**	**41**	**42**	**79**	**76**	**78**	**200**	**185**	**186**	**344**	**343**	**384**	**495**	**491**	**488**	**458**	**465**	**447**
189				**10**	**11**	**10**	**18**	**18**	**18**	**42**	**41**	**42**	**79**	**76**	**78**	**200**	**185**	**187**	**347**	**347**	**386**	**491**	**490**	**489**	**458**	**463**	**447**
192				**10**	**11**	**11**	**18**	**19**	**18**	**42**	**41**	**42**	**79**	**77**	**78**	**202**	**187**	**188**	**346**	**350**	**390**	**494**	**490**	**485**	**454**	**457**	**444**
195				**10**	**11**	**10**	**18**	**18**	**18**	**42**	**41**	**42**	**79**	**77**	**80**	**202**	**187**	**186**	**349**	**353**	**392**	**493**	**490**	**489**	**453**	**456**	**441**
198				**10**	**11**	**11**	**18**	**18**	**19**	**43**	**42**	**43**	**79**	**78**	**78**	**204**	**189**	**189**	**350**	**352**	**391**	**491**	**488**	**485**	**448**	**451**	**443**
201				**11**	**11**	**11**	**18**	**19**	**19**	**42**	**41**	**43**	**80**	**78**	**80**	**204**	**189**	**189**	**351**	**356**	**392**	**488**	**486**	**482**	**448**	**448**	**442**
204				**11**	**11**	**11**	**19**	**18**	**19**	**43**	**42**	**43**	**80**	**78**	**79**	**207**	**190**	**190**	**354**	**356**	**392**	**486**	**485**	**480**	**448**	**445**	**439**
207				**11**	**11**	**11**	**19**	**19**	**19**	**43**	**43**	**44**	**80**	**78**	**79**	**207**	**189**	**190**	**351**	**357**	**394**	**488**	**488**	**482**	**442**	**443**	**439**
210				**11**	**11**	**11**	**19**	**19**	**19**	**44**	**42**	**43**	**80**	**78**	**80**	**208**	**190**	**190**	**354**	**358**	**393**	**485**	**483**	**481**	**443**	**436**	**436**
213				**11**	**11**	**11**	**19**	**19**	**19**	**43**	**42**	**43**	**80**	**77**	**81**	**206**	**189**	**191**	**355**	**357**	**396**	**485**	**483**	**480**	**440**	**440**	**437**
216				**11**	**11**	**11**	**19**	**19**	**19**	**44**	**43**	**44**	**82**	**79**	**80**	**207**	**189**	**190**	**354**	**358**	**395**	**485**	**482**	**481**	**442**	**434**	**436**
219				**11**	**12**	**11**	**19**	**20**	**19**	**43**	**42**	**43**	**80**	**78**	**81**	**207**	**191**	**193**	**355**	**359**	**398**	**485**	**482**	**478**	**440**	**432**	**434**
222				**12**	**12**	**12**	**19**	**20**	**19**	**44**	**43**	**44**	**81**	**79**	**80**	**208**	**191**	**192**	**355**	**362**	**397**	**484**	**483**	**480**	**438**	**431**	**434**

225				**12**	**12**	**12**	**19**	**20**	**19**	**44**	**43**	**44**	**82**	**79**	**81**	**207**	**192**	**193**	**354**	**359**	**396**	**482**	**481**	**476**	**438**	**436**	**433**
228				**11**	**12**	**12**	**20**	**20**	**19**	**44**	**43**	**44**	**81**	**78**	**81**	**209**	**191**	**192**	**355**	**360**	**396**	**485**	**482**	**478**	**434**	**429**	**433**
231				**12**	**12**	**12**	**20**	**20**	**20**	**44**	**43**	**44**	**81**	**80**	**82**	**209**	**193**	**193**	**358**	**359**	**400**	**483**	**479**	**476**	**433**	**427**	**430**

10^6 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
70				**4**	**4**	**4**	**5**	**4**	**4**	**5**	**4**	**6**	**5**	**5**	**5**	**4**	**5**	**5**	**4**	**4**		**5**	**5**	**5**	**4**	**4**	**4**
73				**4**	**4**	**4**	**5**	**5**	**5**	**6**	**4**	**6**	**6**	**6**	**5**	**5**	**5**	**5**	**5**	**4**		**5**	**5**	**5**	**4**	**4**	**4**
76				**4**	**4**	**4**	**5**	**5**	**5**	**6**	**5**	**6**	**6**	**6**	**5**	**5**	**5**	**5**	**5**	**5**		**5**	**5**	**5**	**4**	**5**	**5**
120				**5**	**5**	**5**	**7**	**6**	**6**	**10**	**7**	**10**	**10**	**10**	**9**	**8**	**9**	**9**	**8**	**8**		**9**	**10**	**9**	**8**	**9**	**9**
123				**6**	**6**	**5**	**7**	**6**	**6**	**11**	**8**	**11**	**11**	**12**	**11**	**9**	**10**	**11**	**9**	**9**		**11**	**11**	**10**	**9**	**10**	**10**
126				**6**	**6**	**5**	**7**	**6**	**6**	**11**	**8**	**11**	**12**	**13**	**11**	**10**	**11**	**11**	**10**	**10**		**12**	**12**	**11**	**10**	**11**	**11**
129				**6**	**6**	**5**	**7**	**6**	**6**	**12**	**8**	**12**	**14**	**14**	**12**	**11**	**12**	**13**	**11**	**12**		**14**	**14**	**13**	**12**	**12**	**12**
132				**6**	**6**	**6**	**7**	**6**	**6**	**13**	**9**	**13**	**15**	**16**	**13**	**13**	**13**	**14**	**12**	**13**		**15**	**16**	**14**	**13**	**13**	**13**
135				**6**	**6**	**6**	**7**	**6**	**7**	**13**	**9**	**13**	**16**	**17**	**15**	**13**	**15**	**16**	**13**	**14**		**16**	**18**	**17**	**14**	**15**	**15**
138				**6**	**6**	**6**	**7**	**6**	**7**	**14**	**9**	**14**	**18**		**16**	**15**	**18**	**18**	**15**	**16**		**18**	**20**	**18**	**15**	**16**	**16**
141				**6**	**6**	**6**	**7**	**6**	**6**	**14**	**10**	**14**	**19**	**21**	**19**	**18**	**25**	**21**	**17**	**18**		**21**	**23**	**21**	**18**	**19**	**19**
144				**6**	**6**	**6**	**7**	**6**	**7**	**14**	**10**	**14**	**20**	**22**	**21**	**23**	**26**	**23**	**22**	**20**		**24**	**27**	**24**	**20**	**21**	**21**
147				**6**	**6**	**6**	**8**	**6**	**7**	**15**	**10**	**15**	**22**	**24**	**22**	**27**	**30**	**26**	**27**	**23**		**28**	**30**	**28**	**23**	**25**	**25**
150				**6**	**6**	**6**	**8**	**7**	**7**	**15**	**10**	**16**	**24**	**25**	**24**	**31**	**33**	**29**	**30**	**26**		**31**	**35**	**32**	**27**	**28**	**28**
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156				**7**	**6**	**6**	**8**	**7**	**7**	**16**	**11**	**16**	**27**	**30**	**28**	**39**	**42**	**37**	**40**	**36**		**43**	**48**	**45**	**36**	**40**	**39**
159				**7**	**6**	**6**	**8**	**7**	**7**	**16**	**11**	**16**	**28**	**31**	**29**	**43**	**45**	**40**	**44**	**42**		**50**	**54**	**50**	**42**	**48**	**47**
162				**7**	**7**	**6**	**8**	**7**	**7**	**16**	**11**	**17**	**31**	**32**	**30**	**46**	**51**	**45**	**52**	**50**		**57**	**63**	**58**	**52**	**58**	**58**
165				**7**	**7**	**6**	**8**	**7**	**7**	**16**	**11**	**17**	**31**	**34**	**32**	**50**	**56**	**50**	**58**	**58**		**67**	**71**	**67**	**63**	**70**	**69**
168				**7**	**7**	**6**	**8**	**7**	**7**	**16**	**11**	**17**	**32**	**35**	**32**	**53**	**60**	**53**	**66**	**67**		**75**	**81**	**77**	**74**	**84**	**81**
171				**7**	**7**	**6**	**8**	**7**	**7**	**16**	**11**	**17**	**33**	**36**	**33**	**59**	**66**	**58**	**74**	**77**		**85**	**90**	**88**	**86**	**97**	**97**

174				**7**	**7**	**7**	**8**	**7**	**7**	**16**	**11**	**17**	**34**	**36**	**34**	**62**	**70**	**63**	**81**	**86**		**94**	**100**	**96**	**99**	**111**	**110**
177				**7**	**7**	**7**	**8**	**7**	**8**	**16**	**11**	**17**	**34**	**37**	**35**	**65**	**71**	**66**	**87**	**94**		**101**	**109**	**103**	**110**	**128**	**124**
180				**7**	**7**	**7**	**9**	**7**	**7**	**17**	**11**	**18**	**35**	**38**	**36**	**70**	**77**	**70**	**93**	**103**		**109**	**118**	**114**	**126**	**145**	**141**
183				**7**	**7**	**7**	**9**	**8**	**8**	**17**	**11**	**18**	**35**	**39**	**36**	**74**	**78**	**73**	**99**	**113**		**117**	**128**	**122**	**141**	**164**	**161**
186				**7**	**7**	**7**	**9**	**7**	**7**	**17**	**11**	**18**	**35**	**39**	**36**	**78**	**81**	**78**	**105**	**121**		**122**	**134**	**131**	**155**	**180**	**179**
189				**7**	**7**	**7**	**9**	**8**	**8**	**17**	**12**	**18**	**36**	**39**	**37**	**80**	**84**	**80**	**107**	**127**		**128**	**139**	**138**	**184**	**216**	**202**
192				**8**	**7**	**7**	**9**	**8**	**8**	**18**	**11**	**18**	**36**	**39**	**37**	**80**	**84**	**81**	**113**	**135**		**133**	**148**	**142**	**208**	**242**	**246**
195				**8**	**7**	**7**	**9**	**7**	**8**	**18**	**11**	**19**	**36**	**40**	**38**	**84**	**85**	**83**	**119**	**141**		**141**	**153**	**150**	**234**	**267**	**272**
198				**8**	**8**	**7**	**9**	**8**	**8**	**17**	**12**	**18**	**37**	**41**	**38**	**84**	**86**	**84**	**123**	**148**		**144**	**158**	**154**	**260**	**293**	**297**
201				**8**	**7**	**7**	**9**	**8**	**8**	**18**	**12**	**19**	**37**	**40**	**37**	**85**	**88**	**86**	**127**	**154**		**149**	**163**	**162**	**286**	**318**	**323**
204				**8**	**8**	**7**	**9**	**8**	**8**	**18**	**12**	**19**	**37**	**41**	**38**	**86**	**88**	**87**	**133**	**159**		**153**	**168**	**164**	**312**	**344**	**348**
207				**8**	**8**	**7**	**9**	**8**	**8**	**18**	**12**	**18**	**38**	**40**	**38**	**87**	**89**	**88**	**137**	**164**		**157**	**170**	**170**	**337**	**369**	**374**
210				**8**	**8**	**8**	**9**	**8**	**8**	**17**	**12**	**19**	**37**	**40**	**37**	**86**	**89**	**89**	**140**	**167**		**158**	**172**	**172**	**390**	**394**	**396**
213				**8**	**8**	**7**	**9**	**8**	**8**	**18**	**11**	**19**	**38**	**40**	**38**	**87**	**89**	**89**	**143**	**173**		**160**	**175**	**176**	**412**	**413**	**412**
216				**8**	**8**	**7**	**10**	**8**	**8**	**18**	**11**	**20**	**37**	**41**	**38**	**86**	**89**	**89**	**145**	**176**		**161**	**177**	**176**	**435**	**431**	**430**
219				**8**	**8**	**8**	**10**	**8**	**9**	**18**	**12**	**19**	**37**	**40**	**38**	**86**	**89**	**89**	**147**	**181**		**163**	**179**	**179**	**439**	**439**	**440**
222				**8**	**8**	**8**	**10**	**8**	**9**	**18**	**12**	**19**	**37**	**41**	**38**	**87**	**90**	**90**	**148**	**183**		**164**	**179**	**183**	**431**	**429**	**434**
225				**8**	**8**	**8**	**10**	**8**	**8**	**18**	**12**	**19**	**37**	**41**	**38**	**87**	**90**	**90**	**150**	**186**		**164**	**180**	**184**	**417**	**420**	**427**
228				**8**	**8**	**8**	**10**	**8**	**9**	**19**	**11**	**19**	**38**	**41**	**38**	**86**	**90**	**89**	**151**	**190**		**165**	**179**	**185**	**403**	**420**	**425**
231				**9**	**8**	**8**	**10**	**8**	**9**	**18**	**12**	**19**	**38**	**41**	**39**	**88**	**89**	**90**	**152**	**192**		**167**	**181**	**187**	**400**	**408**	**415**
234				**8**	**8**	**8**	**10**	**9**	**9**	**18**	**12**	**19**	**38**	**41**	**38**	**86**	**88**	**91**	**153**	**196**		**166**	**181**	**187**	**394**	**404**	**411**
237				**8**	**8**	**8**	**10**	**9**	**9**	**18**	**11**	**20**	**37**	**41**	**38**	**85**	**89**	**91**	**152**	**195**		**166**	**183**	**188**	**383**	**402**	**407**
240				**9**	**8**	**8**	**10**	**9**	**9**	**18**	**11**	**20**	**38**	**41**	**39**	**86**	**89**	**90**	**153**	**199**		**167**	**182**	**188**	**373**	**395**	**400**
243				**9**	**8**	**8**	**10**	**9**	**9**	**19**	**12**	**20**	**37**	**41**	**37**	**85**	**89**	**91**	**153**	**201**		**169**	**183**	**190**	**369**	**390**	**396**
246				**9**	**9**	**8**	**10**	**9**	**9**	**19**	**11**	**20**	**39**	**42**	**38**	**86**	**89**	**90**	**154**	**203**		**167**	**183**	**190**	**361**	**388**	**394**
249				**9**	**9**	**8**	**10**	**9**	**9**	**19**	**11**	**20**	**38**	**42**	**39**	**86**	**89**	**91**	**154**	**204**		**167**	**183**	**191**	**355**	**384**	**391**
252				**9**	**9**	**8**	**11**	**9**	**9**	**19**	**11**	**20**	**38**	**41**	**38**	**85**	**88**	**90**	**155**	**204**		**168**	**185**	**191**	**356**	**381**	**386**
255				**9**	**9**	**8**	**11**	**9**	**9**	**19**	**12**	**20**	**38**	**41**	**38**	**85**	**89**	**91**	**154**	**205**		**168**	**183**	**192**	**350**	**377**	**383**
258				**9**	**9**	**8**	**11**	**9**	**9**	**19**	**12**	**20**	**38**	**41**	**39**	**85**	**88**	**90**	**156**	**206**		**169**	**185**	**191**	**348**	**375**	**382**

261				**9**	**9**	**9**	**11**	**9**	**9**	**19**	**12**	**20**	**38**	**41**	**38**	**84**	**88**	**90**	**155**	**207**		**169**	**184**	**190**	**343**	**372**	**380**
264				**9**	**9**	**9**	**11**	**9**	**10**	**19**	**12**	**20**	**38**	**42**	**38**	**84**	**88**	**90**	**156**	**206**		**168**	**186**	**191**	**341**	**371**	**379**

5x10^5 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
181										**8**	**8**	**8**	**21**	**28**	**32**	**30**	**35**	**37**	**5**	**9**	**6**	**21**	**26**	**22**	**36**	**40**	**39**
184										**8**	**8**	**8**	**21**	**29**	**33**	**33**	**39**	**41**	**11**	**17**	**13**	**28**	**33**	**30**	**42**	**48**	**47**
187										**8**	**8**	**8**	**21**	**28**	**33**	**37**	**43**	**45**	**18**	**22**	**18**	**35**	**39**	**35**	**52**	**58**	**58**
190										**8**	**8**	**8**	**21**	**29**	**34**	**40**	**47**	**51**	**26**	**32**	**26**	**42**	**48**	**43**	**63**	**70**	**69**
193										**8**	**8**	**8**	**21**	**27**	**33**	**43**	**53**	**55**	**35**	**40**	**35**	**52**	**56**	**52**	**74**	**84**	**81**
196										**7**	**8**	**8**	**21**	**27**	**34**	**46**	**56**	**60**	**44**	**50**	**46**	**60**	**66**	**62**	**86**	**97**	**97**
199										**8**	**8**	**8**	**21**	**27**	**34**	**50**	**63**	**66**	**53**	**59**	**57**	**70**	**75**	**73**	**99**	**111**	**110**
202										**8**	**8**	**8**	**21**	**27**	**33**	**54**	**66**	**70**	**63**	**68**	**65**	**79**	**85**	**81**	**110**	**128**	**124**
205										**8**	**8**	**8**	**21**	**28**	**34**	**64**	**78**	**82**	**69**	**78**	**72**	**86**	**94**	**88**	**126**	**145**	**141**
208										**8**	**8**	**8**	**21**		**33**	**68**	**83**	**87**	**78**	**86**	**82**	**94**	**103**	**99**	**141**	**164**	**161**
210										**7**	**8**	**8**	**20**	**26**	**33**	**70**	**87**	**92**	**85**	**97**	**91**	**102**	**113**	**107**	**155**	**180**	**179**
214										**8**	**8**	**8**	**21**	**27**	**33**	**80**	**97**	**102**	**91**	**103**	**100**	**107**	**119**	**116**	**170**	**180**	**179**
217										**8**	**8**	**8**	**20**	**26**	**32**	**80**	**99**	**104**	**96**	**108**	**106**	**113**	**124**	**123**	**184**	**202**	**199**
220										**8**	**8**	**8**	**20**	**27**	**32**	**78**	**109**	**105**	**93**	**119**	**117**	**110**	**135**	**133**	**216**	**206**	**193**
223										**8**	**8**	**8**	**21**	**28**	**32**	**85**	**110**	**108**	**99**	**124**	**123**	**116**	**140**	**139**	**232**	**230**	**222**
226										**8**	**8**	**8**	**21**	**29**	**33**	**86**	**112**	**110**	**108**	**126**	**126**	**124**	**142**	**142**	**250**	**242**	**232**
229										**8**	**8**	**8**	**21**	**28**	**33**	**89**	**115**	**113**	**113**	**133**	**132**	**130**	**149**	**149**	**272**	**258**	**245**
232										**8**	**8**	**8**	**21**	**29**	**34**	**89**	**119**	**117**	**116**	**137**	**134**	**132**	**153**	**150**	**292**	**274**	**260**
235										**8**	**8**	**8**	**21**	**27**	**33**	**89**	**120**	**120**	**120**	**143**	**137**	**137**	**159**	**154**	**315**	**292**	**275**
238										**8**	**8**	**8**	**21**	**27**	**34**	**90**	**123**	**122**	**124**	**146**	**141**	**141**	**162**	**157**	**345**	**314**	**291**
241										**8**	**8**	**8**	**21**	**27**	**34**	**89**	**124**	**125**	**125**	**148**	**143**	**141**	**165**	**160**	**372**	**337**	**313**
244										**7**	**8**	**8**	**21**	**27**	**33**	**89**	**125**	**125**	**127**	**151**	**145**	**144**	**168**	**161**	**408**	**368**	**334**

247										**8**	**8**	**8**	**21**	**28**	**34**	**91**	**127**	**127**	**131**	**153**	**147**	**147**	**169**	**163**	**434**	**391**	**362**
250										**8**	**8**	**8**	**21**	**27**	**33**	**89**	**126**	**128**	**132**	**154**	**146**	**148**	**170**	**163**	**458**	**428**	**387**
253										**8**	**8**	**8**	**20**	**26**	**33**	**89**	**127**	**128**	**133**	**156**	**149**	**150**	**173**	**166**	**462**	**458**	**416**
256										**8**	**8**	**8**	**21**	**27**	**33**	**90**	**128**	**130**	**136**	**156**	**149**	**152**	**172**	**166**	**457**	**480**	**444**
259										**7**	**8**	**8**	**20**	**26**	**32**	**89**	**126**	**130**	**135**	**157**	**151**	**151**	**174**	**167**	**455**	**487**	**463**
262										**8**	**8**	**8**	**20**	**26**	**32**	**88**	**127**	**130**	**135**	**156**	**151**	**151**	**173**	**168**	**451**	**485**	**476**
265										**8**	**8**	**8**	**20**	**26**	**32**	**88**	**128**	**131**	**135**	**159**	**152**	**152**	**175**	**169**	**447**	**484**	**474**
268										**8**	**8**	**8**	**21**	**26**	**31**	**88**	**127**	**130**	**134**	**159**	**151**	**150**	**175**	**168**	**445**	**480**	**473**
271										**8**	**8**	**8**	**20**	**26**	**31**	**88**	**127**	**130**	**135**	**158**	**153**	**151**	**175**	**169**	**438**	**475**	**468**
274										**8**	**8**	**8**	**20**	**26**	**31**	**88**	**128**	**132**	**135**	**160**	**154**	**152**	**176**	**170**	**438**	**471**	**467**
277										**8**	**8**	**8**	**20**	**26**	**30**	**88**	**127**	**131**	**135**	**161**	**154**	**151**	**178**	**170**	**433**	**470**	**465**
280										**8**	**8**	**8**	**20**	**25**	**31**	**88**	**127**	**131**	**134**	**161**	**154**	**150**	**178**	**170**	**431**	**465**	**462**
283										**8**	**8**	**8**	**20**	**25**	**29**	**87**	**127**	**130**	**132**	**156**	**152**	**149**	**173**	**168**	**423**	**455**	**451**
286										**8**	**8**	**8**	**20**	**25**	**29**	**88**	**125**	**130**	**130**	**157**	**153**	**146**	**174**	**169**	**423**	**456**	**452**
289										**8**	**8**	**8**	**20**	**25**	**29**	**88**	**126**	**131**	**131**	**157**	**152**	**147**	**173**	**168**	**420**	**455**	**449**
292										**8**	**8**	**8**	**19**	**25**	**30**	**89**	**127**	**131**	**131**	**159**	**152**	**148**	**176**	**169**	**417**	**449**	**448**
295										**8**	**8**	**8**	**20**	**25**	**29**	**88**	**125**	**132**	**127**	**157**	**153**	**144**	**173**	**170**	**414**	**446**	**444**
298										**8**	**8**	**8**	**20**	**25**	**29**	**88**	**125**	**131**	**126**	**156**	**154**	**143**	**173**	**170**	**411**	**444**	**442**
301										**8**	**8**	**8**	**20**	**24**	**28**	**88**	**127**	**132**	**127**	**159**	**152**	**143**	**175**	**169**	**409**	**441**	**441**
304										**8**	**8**	**8**	**20**	**25**	**29**	**86**	**125**	**129**	**125**	**159**	**155**	**141**	**175**	**171**	**408**	**439**	**437**
307										**8**	**8**	**8**	**20**	**25**	**29**	**86**	**125**	**130**	**125**	**157**	**153**	**141**	**174**	**169**	**403**	**438**	**436**
310										**8**	**8**	**8**	**20**	**25**	**29**	**86**	**126**	**132**	**125**	**157**	**153**	**141**	**173**	**170**	**403**	**436**	**437**
313										**8**	**8**	**8**	**20**	**25**	**29**	**87**	**126**	**131**	**123**	**156**	**154**	**140**	**172**	**170**	**402**	**435**	**433**
316										**8**	**8**	**8**	**20**	**24**	**28**	**86**	**126**	**130**	**124**	**156**	**153**	**140**	**172**	**169**	**400**	**434**	**432**
319										**8**	**8**	**8**	**20**	**24**	**28**	**87**	**126**	**131**	**123**	**158**	**152**	**139**	**174**	**169**	**399**	**432**	**430**
322										**8**	**8**	**8**	**20**	**24**	**28**	**86**	**126**	**131**	**122**	**156**	**153**	**138**	**172**	**169**	**399**	**429**	**429**
325										**8**	**8**	**8**	**19**	**24**	**29**	**86**	**125**	**130**	**121**	**156**	**154**	**137**	**172**	**170**	**395**	**430**	**430**
328										**8**	**8**	**8**	**19**	**24**	**28**	**87**	**128**	**130**	**121**	**157**	**153**	**137**	**173**	**170**	**394**	**423**	**427**
331										**8**	**8**	**8**	**20**	**24**	**27**	**87**	**126**	**130**	**119**	**155**	**154**	**135**	**172**	**170**	**392**	**427**	**425**

334										**8**	**8**	**8**	**20**	**24**	**28**	**86**	**125**	**131**	**119**	**155**	**154**	**136**	**171**	**170**	**392**	**422**	**422**
337										**8**	**8**	**8**	**19**	**25**	**28**	**87**	**127**	**132**	**121**	**157**	**153**	**137**	**173**	**170**	**388**	**419**	**422**
340										**8**	**8**	**8**	**19**	**24**	**27**	**86**	**128**	**131**	**119**	**156**	**154**	**135**	**172**	**171**	**389**	**417**	**419**
343										**8**	**8**	**8**	**20**	**25**	**28**	**86**	**126**	**131**	**117**	**154**	**153**	**133**	**171**	**170**	**387**	**418**	**417**
346										**8**	**8**	**8**	**20**	**25**	**28**	**86**	**126**	**131**	**117**	**154**	**153**	**133**	**171**	**170**	**387**	**418**	**417**
349										**8**	**9**	**8**	**19**	**24**	**28**	**87**	**126**	**132**	**116**	**155**	**152**	**133**	**171**	**168**	**382**	**415**	**416**
352										**8**	**9**	**8**	**19**	**24**	**28**	**87**	**126**	**132**	**116**	**155**	**152**	**133**	**171**	**168**	**382**	**415**	**416**
355										**8**	**9**	**8**	**19**	**24**	**28**	**87**	**126**	**132**	**116**	**155**	**152**	**133**	**171**	**168**	**382**	**415**	**416**
358										**8**	**9**	**8**	**19**	**24**	**28**	**87**	**126**	**132**	**116**	**155**	**152**	**133**	**171**	**168**	**382**	**415**	**416**
361										**8**	**9**	**8**	**19**	**24**	**28**	**87**	**126**	**132**	**116**	**155**	**152**	**133**	**171**	**168**	**382**	**415**	**416**

10^5 cfu/ml (0 min)																												
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
220										**6**	**6**	**5**	**14**	**13**	**13**	**26**	**25**	**25**	**30**	**27**	**29**	**25**	**26**	**26**	**31**	**26**	**25**
223										**6**	**5**	**5**	**15**	**15**	**14**	**30**	**27**	**26**	**33**	**33**	**35**	**31**	**32**	**32**	**36**	**31**	**32**
226										**6**	**6**	**6**	**16**	**15**	**15**	**32**	**30**	**28**	**37**	**36**	**37**	**33**	**34**	**35**	**40**	**34**	**34**
229										**6**	**5**	**6**	**16**	**16**	**16**	**35**	**33**	**31**	**40**	**41**	**41**	**37**	**38**	**37**	**44**	**37**	**39**
232										**6**	**5**	**5**	**16**	**17**	**16**	**39**	**37**	**33**	**46**	**46**	**46**	**40**	**42**	**42**	**52**	**44**	**45**
235										**6**	**5**	**6**	**17**	**17**	**17**	**41**	**39**	**36**	**51**	**49**	**49**	**45**	**46**	**45**	**56**	**49**	**50**
238										**5**	**5**	**5**	**18**	**17**	**17**	**44**	**41**	**38**	**57**	**53**	**54**	**51**	**51**	**50**	**64**	**54**	**54**
241										**6**	**5**	**5**	**18**	**17**	**17**	**46**	**43**	**41**	**60**	**58**	**57**	**55**	**56**	**54**	**72**	**60**	**61**
244										**6**	**5**	**5**	**18**	**18**	**17**	**48**	**44**	**43**	**65**	**60**	**62**	**58**	**62**	**60**	**79**	**66**	**67**
247										**6**	**6**	**5**	**18**		**17**	**51**	**46**	**45**	**69**	**64**	**66**	**63**	**66**	**63**	**88**	**73**	**73**
250										**6**	**5**	**5**	**17**	**18**	**17**	**52**	**47**	**46**	**75**	**70**	**70**	**67**	**71**	**68**	**96**	**82**	**81**
253										**5**	**5**	**5**	**18**	**18**	**17**	**54**	**48**	**47**	**80**	**74**	**75**	**73**	**77**	**73**	**109**	**89**	**90**
256										**5**	**6**	**5**	**17**	**18**	**17**	**55**	**50**	**49**	**84**	**77**	**78**	**75**	**81**	**76**	**118**	**100**	**96**
259										**6**	**6**	**5**	**18**	**18**	**17**	**57**	**50**	**50**	**87**	**80**	**81**	**80**	**86**	**81**	**131**	**109**	**105**

262										**6**	**5**	**5**	**18**	**17**	**17**	**58**	**52**	**50**	**90**	**84**	**84**	**83**	**90**	**86**	**143**	**118**	**116**
265										**6**	**6**	**5**	**17**	**17**	**17**	**61**	**53**	**52**	**95**	**87**	**88**	**87**	**96**	**87**	**155**	**132**	**127**
268										**6**	**5**	**5**	**17**	**17**	**16**	**60**	**54**	**52**	**97**	**90**	**91**	**91**	**100**	**93**	**168**	**146**	**139**
271										**6**	**5**	**5**	**17**	**17**	**16**	**60**	**54**	**53**	**99**	**91**	**91**	**94**	**107**	**97**	**186**	**153**	**152**
274										**6**	**6**	**5**	**17**	**17**	**16**	**61**	**55**	**54**	**103**	**94**	**97**	**97**	**112**	**100**	**201**	**166**	**165**
277										**6**	**5**	**5**	**17**	**17**	**16**	**62**	**55**	**54**	**104**	**97**	**97**	**99**	**113**	**102**	**222**	**180**	**183**
280										**6**	**5**	**5**	**17**	**17**	**16**	**62**	**55**	**54**	**106**	**99**	**99**	**104**	**118**	**105**	**238**	**198**	**198**
283										**6**	**5**	**5**	**17**	**17**	**16**	**63**	**56**	**54**	**108**	**100**	**101**	**106**	**123**	**108**	**265**	**220**	**220**
286										**6**	**5**	**5**	**17**	**16**	**15**	**63**	**56**	**55**	**107**	**102**	**102**	**109**	**127**	**113**	**293**	**235**	**241**
289										**6**	**5**	**5**	**17**	**17**	**16**	**63**	**56**	**55**	**108**	**102**	**102**	**110**	**131**	**116**	**316**	**254**	**258**
292										**6**	**6**	**5**	**16**	**16**	**16**	**65**	**57**	**56**	**109**	**102**	**104**	**111**	**134**	**118**	**348**	**277**	**281**
295										**6**	**5**	**5**	**17**	**17**	**16**	**64**	**56**	**56**	**109**	**103**	**104**	**112**	**139**	**119**	**376**	**302**	**311**
298										**6**	**5**	**5**	**16**	**16**	**15**	**64**	**56**	**56**	**110**	**103**	**104**	**113**	**141**	**121**	**413**	**330**	**343**
301										**6**	**6**	**5**	**16**	**16**	**15**	**64**	**56**	**56**	**110**	**103**	**104**	**112**	**145**	**121**	**446**	**362**	**376**
304										**6**	**6**	**6**	**16**	**16**	**15**	**65**	**57**	**56**	**110**	**105**	**105**	**113**	**146**	**124**	**477**	**396**	**413**
307										**6**	**6**	**5**	**16**	**16**	**15**	**63**	**56**	**55**	**110**	**103**	**105**	**114**	**148**	**123**	**492**	**430**	**451**
310										**6**	**6**	**5**	**16**	**16**	**15**	**64**	**57**	**55**	**110**	**105**	**106**	**114**	**150**	**124**	**488**	**454**	**477**
313										**6**	**5**	**6**	**16**	**16**	**15**	**65**	**56**	**56**	**110**	**104**	**105**	**114**	**152**	**125**	**488**	**467**	**484**
316										**6**	**6**	**6**	**16**	**16**	**15**	**65**	**57**	**56**	**110**	**105**	**106**	**116**	**154**	**125**	**488**	**471**	**486**
319										**6**	**6**	**5**	**16**	**16**	**15**	**64**	**57**	**56**	**111**	**105**	**106**	**115**	**154**	**126**	**485**	**472**	**484**
322										**6**	**5**	**6**	**16**	**16**	**15**	**65**	**56**	**56**	**111**	**104**	**106**	**115**	**156**	**126**	**484**	**470**	**481**
325										**6**	**6**	**6**	**16**	**16**	**15**	**64**	**57**	**56**	**112**	**105**	**105**	**114**	**157**	**126**	**484**	**463**	**479**
328										**6**	**5**	**6**	**16**	**16**	**15**	**65**	**56**	**55**	**112**	**105**	**106**	**114**	**158**	**127**	**480**	**460**	**475**
331										**6**	**6**	**5**	**16**	**16**	**15**	**64**	**56**	**56**	**111**	**104**	**107**	**115**	**160**	**126**	**482**	**459**	**473**
334										**6**	**5**	**6**	**16**	**16**	**15**	**64**	**56**	**55**	**111**	**105**	**105**	**115**	**159**	**127**	**480**	**456**	**469**
337										**6**	**5**	**6**	**17**	**16**	**15**	**64**	**57**	**55**	**111**	**105**	**105**	**114**	**159**	**126**	**477**	**450**	**462**
340										**6**	**6**	**5**	**16**	**16**	**14**	**64**	**57**	**55**	**111**	**104**	**106**	**115**	**160**	**126**	**477**	**447**	**458**
343										**6**	**5**	**6**	**16**	**16**	**15**	**64**	**56**	**55**	**111**	**105**	**106**	**114**	**159**	**128**	**473**	**445**	**456**
346										**6**	**5**	**6**	**16**	**16**	**15**	**64**	**56**	**55**	**111**	**105**	**106**	**114**	**159**	**128**	**473**	**445**	**456**

349										**6**	**6**	**6**	**16**	**16**	**15**	**66**	**57**	**55**	**111**	**105**	**106**	**115**	**159**	**127**	**472**	**441**	**452**
352										**6**	**6**	**6**	**16**	**16**	**15**	**66**	**57**	**55**	**111**	**105**	**106**	**115**	**159**	**127**	**472**	**441**	**452**
355										**6**	**6**	**6**	**16**	**16**	**15**	**66**	**57**	**55**	**111**	**105**	**106**	**115**	**159**	**127**	**472**	**441**	**452**
358										**6**	**6**	**6**	**16**	**16**	**15**	**66**	**57**	**55**	**111**	**105**	**106**	**115**	**159**	**127**	**472**	**441**	**452**
361										**6**	**6**	**6**	**16**	**16**	**15**	**66**	**57**	**55**	**111**	**105**	**106**	**115**	**159**	**127**	**472**	**441**	**452**

1000 NTU 10^7 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
3				**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
6				**2**	**2**	**2**	**3**	**2**	**2**	**3**	**3**	**3**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**3**	**3**
9				**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
12				**3**	**3**	**3**	**4**	**3**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**	**3**	**4**	**4**	**4**
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2000 NTU 10^7 cfu/ml (0 min)																											
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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96				**12**	**13**	**12**	**50**	**59**	**55**	**114**	**84**	**73**		**84**	**76**		**82**	**66**	**114**	**88**	**63**	**154**	**100**	**86**	**188**	**118**	**137**
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milk 10^7 cfu/ml (0 min)																										
time (min)	**5x10^8 pfu/ml**			**5x10^7 pfu/ml**			**5x10^6 pfu/ml**			**5x10^5 pfu/ml**			**5x10^4 pfu/ml**			**5x10^3 pfu/ml**			**5x10^2 pfu/ml**			**5x10^1 pfu/ml**			**0 pfu/ml**		
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93				**6**	**7**	**6**	**26**	**25**	**23**	**57**	**65**	**49**	**85**	**84**	**90**	**109**	**97**	**101**	**129**	**111**	**107**	**125**	**105**	**116**	**155**	**183**	**163**
96				**6**	**7**	**6**	**27**	**26**	**24**	**59**	**68**	**54**	**98**	**97**	**104**	**129**	**115**	**124**	**157**	**130**	**127**	**151**	**129**	**140**	**184**	**219**	**191**
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111				**7**	**7**	**7**	**31**	**30**	**28**	**74**	**85**	**70**	**153**	**186**	**194**	**256**	**236**	**231**	**383**	**284**	**261**	**390**	**293**	**371**	**398**	**402**	**375**
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117				**7**	**7**	**7**	**32**	**31**	**30**	**79**	**89**	**74**	**186**	**218**	**223**	**333**	**310**	**311**	**415**	**367**	**356**	**418**	**374**	**418**	**426**	**424**	**396**
120				**7**	**7**	**7**	**33**	**32**	**31**	**81**	**91**	**75**	**191**	**236**	**240**	**372**	**352**	**361**	**428**	**384**	**390**	**428**	**389**	**431**	**437**	**429**	**410**
123				**7**	**7**	**7**	**34**	**33**	**32**	**83**	**94**	**77**	**218**	**250**	**255**	**389**	**380**	**408**	**431**	**397**	**402**	**437**	**403**	**440**	**441**	**435**	**419**
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