doi:10.2533/chimia.2025.644 Chimia 79 (2025) 644–649 © C. Jungo, S. Garcia, J.-F. Brunet, G. Resch

Optimizing the Production of Therapeutic Bacteriophages Through Quality by Design: A Case Study on *Pseudomonas Aeruginosa*

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Abstract: A Failure Modes and Effects Analysis (FMEA) risk assessment was conducted to evaluate and document the criticality of process parameters and material attributes involved in a *Pseudomonas aeruginosa* phage production process. This assessment was carried out following the principles of Quality by Design (QbD) as outlined by the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use. By systematically identifying and controlling critical factors, this approach contributes to the development of a more robust and reproducible phage production process, ultimately enhancing process efficiency and product quality.

Keywords: Antimicrobial resistance · Bacteriophages · Phage therapy · Process characterization · *Pseudomonas aeruginosa* · Quality by Design

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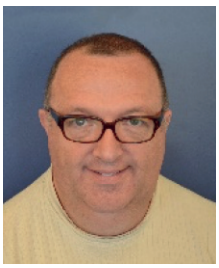

Dr. Jean-François Brunet and his team developed a phage manufacture pipeline in close collaboration with teams at CHUV from the Center for Research and Innovation in Clinical Pharmaceutical Sciences (CRISP, Dr. Grégory Resch, project partner), the Service of Infectious Diseases (Prof. Benoît Guery), and the Pulmonology Service (Prof. Christophe von Garnier). On June 23, 2023, the CHUV was officially recognized by Swissmedic as the first academic institution in the world authorized to manufacture phages under GMP (EudraGMP certificate N°GMPE-CH-1004487).

Dr. Gregory Resch is MER I at the Lausanne University Hospital (CHUV) and Head of the Laboratory of Bacteriophages and Phage Therapy which he founded in 2021 in the Center for Research and Innovation in Clinical Pharmaceutical Sciences (CRISP). For over 25 years, his research has focused on the therapeutic development of bacteriophages (phage therapy) and phage lysins. A principal investigator at the NCCR Microbiomes, Dr. Resch aims to combat multidrug-resistant bacteria while preserving the patient's microbiota. His team developed a phage production pipeline, which has been implemented at the Cell Production Center (CPC). This enabled CHUV to obtain GMP authorization for phage manufacture from Swissmedic, representing a world first for an academic institution. Recipient of numerous grants and awards, he was co-awarded the 2024 Leenaards Foundation prize for translational medical research for a project on personalized phage therapy for respiratory infections.

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1. Introduction

Antimicrobial resistance (AMR) is ranked 5th among the ten major global public health threats according to the World Health Organization (WHO).[1] In the European Union (EU), bacterial AMR causes 35,000 deaths each year, and an annual cost due to healthcare expenditures and productivity losses of €1.5 billion.[2] The spread of drug-resistant pathogens is calling into question our ability to manage even current common bacterial infections with existing antibiotics. Of particular concern is the fact that, without these drugs, routine surgical procedures are becoming increasingly risky, and previously benign infections are once again killing.[3]

New treatments for bacterial infections are therefore urgently needed. Bacteriophages (phages) are ubiquitous and strain-specific for bacterial viruses. Since their discovery in the late 1910's, strictly lytic phages have been used for treating bacterial infections. The advent of antibiotics and the complexity of selecting phages due to their strong specificity relegated phage therapy to oblivion in the 60s and 70s. As part of a response to the antibiotic resistance crisis, there has been a revival of interest in phage therapy in the early 2000s.[4-8] Phages have recently been classified as standardized medicinal biological products, including by Swissmedic. Unfortunately, the absence of data from Randomized Control Trials (RCTs) documenting phage efficacy and safety data currently limits access to Phage Therapy Medicinal Products (PTMP). Accordingly, very recently, six clinical trials have been launched in various countries around the world (Canada, Uzbekistan, United States) and 18 clinical trials are reported to be completed (clinicaltrials.gov), but most patients are still treated on a compassionate basis with magistral preparations.[9] BiomX recently announced positive topline results from its Phase 1b/2a clinical trial evaluating BX004, a phage cocktail targeting *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF) who suffer from chronic lung infections.[10,11] Additional RCTs are therefore eagerly awaited, to enable the marketing authorization of PTMPs and considerably broaden access to this innovative therapy. Some of the keys to large-scale implementation of phage therapy rely on our ability to standardize phage selection protocols (phagograms) and production according to Good Manufacturing Practice (GMP) and improve formulation. In the production and formulation processes, many steps still need to be optimized and standardized. Amongst those, achieving high yields and purity levels remains a challenge.[12] The complexity relies in the amplification of the selected therapeutic phage to be produced on a susceptible bacterial strain (production strain) and then to eliminate or significantly reduce the bacterial debris, proteins, DNA, and endotoxins (Gram negative) that represent potentially harmful contaminants, without altering phage integrity and yield. Therefore, while producing phages is quite straightforward in R&D, refining the protocols to meet the stringent GMP requirements for therapeutic application represents a significant challenge.

Consequently, robust, scalable, and regulatory-compliant processes for therapeutic phage production and purification are urgently needed to ensure the successful implementation of phage therapy in the clinic and its extension to as many applications as possible. To achieve this, a Quality by Design (QbD) approach is crucial for comprehensive process characterization,[13] enabling the identification and control of critical process parameters that influence product quality. By systematically assessing variability and optimizing process conditions, QbD ensures consistency, reproducibility, and compliance with regulatory expectations, ultimately facilitating the large-scale production of safe and effective phage therapies.

Our goal is to characterize and optimize the *P. aeruginosa* phage production process developed by the CHUV (Centre Hospitalier Universitaire Vaud), more precisely by the Laboratory of Bacteriophages and Phage Therapy of Dr. Grégory Resch and Cell Production Center of Dr. Jean-François Brunet, by following a systematic QbD approach according to current health authority expectations (ICH guidelines).

This article outlines the Failure Mode and Effects Analysis (FMEA) risk assessment that was performed to evaluate the criticality of process variables, including process parameters (PP) and material attributes (MA). This risk assessment will serve as the basis for the characterization and optimization studies of CHUV's *P. aeruginosa* phage production process.

1.1 *Pseudomonas Aeruginosa as a Model System*

P. aeruginosa is a gram-negative opportunistic and versatile pathogen. Highly resistant to conventional antibiotic treatment, it often causes nosocomial infections, and it is the main pathogen involved in lung infection of cystic fibrosis (CF) patients.[14] CF is a life-limiting genetic disease that leads to chronic lung infection, requiring almost daily antibiotic treatment. Such intensive antibiotic exposure is very often accompanied by an increase in antibiotic resistance of the pathogen involved, which complicates treatment and ultimately leads to a therapeutic dead-end.[14] Two recent reviews came to similar conclusion that phage therapy represents a promising complimentary strategy to antibiotics for the management of CF lung infections.[15,16] Several hundreds of bacteriophages specific for *P. aeruginosa* have been isolated and published by several research groups in the world[4] and the laboratory of Dr. Resch owns a well-characterized collection of >100 different *P. aeruginosa* phages.

2. FMEA Risk Assessment

QbD is a structured development approach that starts with predefined objectives and focuses on gaining a thorough understanding of the product and process, alongside implementing effective process controls. This approach is grounded in scientific principles and quality risk management. The principles and methodologies detailed in ICH Q8(R2), ICH Q9, ICH Q10, and ICH Q11 are applied during product development to design, optimize, and characterize robust, high-yielding, and transferable processes that consistently deliver products meeting established quality standards.

The QbD approach begins with definition of the Quality Target Product Profile (QTPP), followed by the identification of potential Critical Quality Attributes (CQAs) and Process Performance Attributes (PPAs).

The CQAs and PPAs of the studied *P. aeruginosa* phage production process were based on the framework of requirements for PTMPs outlined in the European Pharmacopeia 11.6, general chapter 5.31 01/2025 © Council of Europe.

2.1 *Critical Quality Attributes (CQAs) and Process Performance Attributes (PPAs)*

According to the European Pharmacopeia 11.6 for PTMPs, general chapter 5.31 01/2025 © Council of Europe, the following CQAs were taken into consideration in our FMEA risk assessment:

- **Identity** (by full DNA sequencing)
- **Microbiological quality** (**Bioburden** level during process; **Sterility** on final product)
- Host-cell impurities and contaminants:
  - **Bacterial endotoxin**
  - **Host-cell Proteins** (by LC-MS)
  - **Host-cell DNA** (by full DNA sequencing)
  - **Temperate phages** (by full DNA sequencing)
- **Appearance** of final product (visual control)
- **pH** of final product

The CQAs Bioburden and Sterility were assessed only in instances where a defined procedure exists to remove or reduce them. These CQAs carry a risk of introduction through accessory solutions, materials, or equipment. However, this risk is expected

to be adequately mitigated by established GMP procedures, such as raw material testing, room qualification, and gowning and handling protocols. As a result, these risks were not addressed within this FMEA.

Also the following PPA was considered:

- **Potency: Phage titer** in pfu/mL

2.2 *Material Attributes (MAs)*

The assessment of material attributes (MAs) was carried out with consideration of each material's intended role within the process. While variability in the MAs was not explicitly evaluated in relation to its impact on CQAs or PPAs, a comprehensive approach was employed to estimate the potential extent of its influence. If a MA was found to have a significant impact on a CQA, it was classified as a Critical Material Attribute (CMA). Conversely, if the impact was primarily on a PPA, the attribute was categorized as a MA.

2.3 *FMEA Methodology*

When sufficient process knowledge and process data is available, FMEA is the preferred risk assessment tool during development studies. This method provides a science- and risk-based framework for evaluating the relationships between process inputs (PPs and MAs) and outputs. It focuses on identifying which CQAs and PPAs are affected at each unit operation. FMEA can be applied to assess the risk associated with both PPs and MAs, and to classify the PPs and MAs.

As defined in ICH Q8(R2), a PP whose variability has an impact on a CQA and therefore should be monitored or controlled to ensure the process produces the desired quality is called a **Critical Process Parameter (CPP).** A PP whose variability has a negligible impact on CQAs but whose variability has an impact on PPAs should be monitored or controlled to ensure the process produces the desired performance (titer, yield) is designated as a **Key Process Parameter (KPP).** Finally, a **General Process Parameter (GPP)** is a PP whose variability has negligible impact on CQAs and PPAs but is still important for process consistency and robustness.

In a similar way, a **Critical Material Attribute (CMA)** is a material (*e.g.* raw materials, buffers, media, processing aids, chromatography resins, viral inactivation additives, starting material, intermediates, packaging material) with attributes whose variability has an impact on a CQA and should be monitored or controlled to ensure the process produces the desired quality.

Each process PP or MA was quantitatively assessed for risk by assigning a numerical score (1, 2, 3, 4) based on the severity (SEV), likelihood of occurrence (OCC), and detectability (DET) of potential failure (Table 1). In the context of a focused FMEA – aimed at identifying potential CPPs and potential CMAs – it is essential to narrow the definition of 'failure mode'. Reasonable excursion outside of the normal operation range (NOR) corresponding to two times the NOR (distance between the target and upper/lower limit multiplied by 2) were considered for the failure mode assessment. If the NOR was not defined and only a target value was described for a process variable in the current description of the production process, a +/– 20% deviation from the target was considered for failure mode assessment.

The Risk Priority Number (RPN) was calculated for each process variable as the product of severity, likelihood of occurrence, and detectability of potential failure (RPN = SEV x OCC x DET). Higher RPN values indicate process variables that may need closer monitoring or adjustment to minimize the risk of failure.

It is important to distinguish between high general risk (as determined by RPN), high risk to CQAs (as determined by Severity to CQAs) and high risk to PPAs (as determined by Severity to PPAs). A high RPN (above a defined threshold as defined in Table 2) indicates a high-risk process variable which requires further risk mitigation in terms of process or material control.

Data to inform on the criticality of process variables was accumulated from the available process development studies.

2.3.1 Scoring process parameters

Each process parameter was assessed for the severity, likelihood of occurrence, and detectability of potential failure and scored according to the scoring system described in Table 1. The following questions were answered:

Severity (SEV): How critical are the consequences of operating outside of the NOR?

Occurrence (OCC): How likely or how often is the failure expected to happen?

Detectability (DET): What is the likelihood that the existing in-process tests will identify the failure?

2.3.2 Interpretation of process parameter scoring outcomes

The following approach was applied for the identification of potential CPPs and KPPs:

- Parameters with a severity score ≥ 3, based on their impact on CQAs, were classified as potential CPPs, regardless of their RPN value. These CPPs will undergo further experimental evaluation during process characterization studies.
- Parameters with a severity score ≥ 3, due to their impact on PPAs, were identified as potential KPPs, independent of their RPN value. These KPPs will also be subject to additional ex-

Table 1: Scorings (1-2-3-4) for process parameters in FMEA.

SEV	**None (1)**	**Low (2)**	**Moderate (3)**	**High (4)**
	No impact to CQAs. No impact to PPAs.	Minor impact to CQAs: still within specifications. Low impact to PPAs.	Moderate impact to CQAs: still within specifications. Moderate impact to PPAs.	High impact to CQAs: Product expected to fail specifications. High impact to PPAs.
OCC	**Very Low (1)**	**Low (2)**	**Moderate (3)**	**High (4)**
	The probability of failure is very low, based on current process knowledge,or no failure to date.	The probability of failure is low, based on current process knowledge, or no failure to date.	The probability of failure is moderate, based on current process knowledge, or at least one observed failure to date.	The probability of failure is high based on repeated failures to date.
DET	**High (1)**	**Moderate (2)**	**Low (3)**	**None (4)**
	Automation or process outputs provide detection of both the failure (*e.g.* through alarms) and its impact at this process step (*e.g. via* appropriate in-process tests)	Automation or process outputs are implemented to detect the failure or its impact at this process step by appropriate in-process tests	No alarms to detect failure, and its impact may not be detected by the process outputs at this step, but prior to product release.	No alarms or systems to detect failure, and its impact may not be detected until product release.

Table 2: RNP scores and recommended actions.

RPN	Actions required
1–6	Low risk to process control: no further action needed.
9–12	Moderate process control risk: additional mitigation measures should be evaluated and documented.
18–64	High process control risk: additional mitigation measures are required.

perimental assessment during process characterization activities.

- Parameters with a RPN exceeding a defined threshold (Table 2) are considered to pose a process control risk. These parameters should be reviewed to assess the potential for additional control measures. While further control may not always be feasible, its applicability should be carefully evaluated and appropriately justified.

2.3.3 Scoring material attributes

It is essential to thoroughly understand the quality attributes of materials. For purchased materials, the supplier's quality management system must be verified to ensure that critical attributes consistently meet the defined acceptance criteria. In line with GMP expectations, raw materials must be properly tested and approved before use. However, even when materials meet their acceptance criteria and are well-controlled, variability within these limits- or the presence of attributes not covered by the acceptance criteria (*e.g.* components of culture media, specifications of chromatography resins) - may still require experimental assessment. This is necessary to evaluate whether such variability could have a significant impact on a CQA or PPA.

Each MA was assessed for severity, likelihood of occurrence, and detectability of potential failure and scored according to the scoring system described in Table 3.

2.3.4 Interpretation of MAs scoring outcomes

The following approach was applied for the assignment of potential CMA:

MAs with a severity score ≥ 3 based on their impact on CQAs, were classified as potential CMAs regardless of their RPN value. These MAs require further investigation to determine whether the risk can be mitigated, for example with experimental studies to characterize variability on process outputs.

MAs with a RPN exceeding a defined threshold (Table 2) are considered to pose a process control risk. These MAs should be reviewed to assess the potential for additional control measures.

3. FMEA Outcome

The FMEA risk assessment for the process variables of the **phage amplification step** is described below and outlined in Table 4 as an example.

The **volume of preculture** added to the production culture is not expected to have an influence on CQAs. If outside of NOR, it could impact the duration of the production culture, as the initial bacterial cell concentration can influence the time necessary to reach the target cell concentration for phage addition. The obtained phage titer at the phage amplification step should not be impacted by this process variable. For these reasons, the scores for SEV were rated at 1. There is a very low likelihood of failure (OCC = 1) as the inoculum volume is measured using a serological graduated pipette. DET was rated at 3 as there is no alarm to detect failure.

The **volume of medium** is not expected to have an influence on CQAs. If outside of NOR, no significant impact on the phage titer is expected. The scores for SEV were rated at 1. There is a very low likelihood of failure (OCC = 1) since all media solutions are mixed using serological graduated pipettes, ensuring consistent measurements during the preparation process. DET was rated at 3 as there is no alarm to detect failure. A failure would however be detected visually during the performance of this step.

The **pH** and the **temperature** are maintained constant during the culture production in the bioreactor. pH is maintained at the target value by adding a base. If the pH or the temperature deviate from NOR, no impact on CQAs is expected. However, the phage titer might be decreased, as bacteria have an optimal pH and temperature for their metabolism. The scores for SEV on CQAs were rated at 1, and on PPA as 3. There is a very low likelihood of failure (OCC = 1) as the pH and the temperature are controlled

Table 3: Scoring (1-2-3-4) for material attributes in FMEA.

SEV	**None (1)**	**Low (2)**	**Moderate (3)**	**High (4)**
	No product contact. No impact to quality attributes. No/low impact to performance attributes. Product expected to meet specifications.	Product contact, but impact on product quality is unlikely or acceptable. Low/moderate impact to performance attributes. Product expected to meet specifications.	Product contact and impact on product quality is possible, but moderate, such that the product specifications may be met.	Product contact and impact on product quality is very likely. When failure occurs, significant impact to quality attributes, and product is expected to fail specifications.
OCC	**Very Low (1)**	**Low (2)**	**Moderate (3)**	**High (4)**
	The probability of failure is very low, based on current process knowledge or no failure to date. Requirements are defined and certified, well-established component.	The probability of failure is low, based on current knowledge and no failure to date. Components are new or not well-established.	The probability of failure is moderate, based on at least one observed excursion to date.	The probability of failure is high, based on multiple observed failures to date.
DET	**High (1)**	**Moderate (2)**	**Low (3)**	**None (4)**
	High likelihood that in-process tests will detect the impact of MA variability on process performance or product quality at the step where the MA is used.	High likelihood that in-process tests will detect the impact of MA variability on process performance or product quality, but only at a later stage, making it difficult to trace the root cause back to the MA.	High likelihood that intermediate or final product tests will detect the impact of MA variability on quality, but lack of in-process detection makes it nearly impossible to trace the root cause to the MA.	Neither in-process nor release tests can detect the impact on process performance or product quality.

automatically. pH is controlled and maintained by an analytical probe, control software, and the addition of base by a peristaltic pump. Temperature is controlled in the bioreactor using a resistance heater for heating and a cooling finger. DET was rated at 1 as pH and temperature are both continuously monitored.

The **stirring speed** is not expected to have an influence on CQAs, but it could impact the phage titer. The scores for SEV on CQAs were rated at 1, and on PPA as 3. It was in fact observed that depending on the stirring speed, foam formation can lead to reduced phage titer. Additionally, insufficient stirring could limit oxygen transfer, potentially slowing down the growth of bacteria, while excessive stirring may increase foam formation, which can negatively affect phage titer. OCC and DET were rated at 1.

The likelihood of failure is minimal, as the stirring speed is regulated by the bioreactor's automated control system, which continuously monitors agitation and dynamically adjusts the power input to maintain a stable stirring rate. This precise control minimizes fluctuations that could impact oxygen transfer, foam generation, and overall culture performance. The risk that a failure is not detected is also minimal since the stirring speed is continuously monitored and alarmed.

Although the **antifoam concentration** is not expected to impact the CQAs, this PP may influence the final phage titer. In the event of foam formation during cultivation, a residue ring tends to accumulate on the bioreactor glass wall just above the liquid surface. This ring is hypothesized to be highly enriched in bacteriophage particles.

For the **volume of phage inoculum**, CQAs are not expected to be affected when operating outside the NOR. While no significant impact on phage titer is anticipated, a reduced phage inoculum volume may prolong the time required to achieve host cell lysis. Conversely, exceeding the NOR for phage suspension volume could lead to a shorter latency period and potentially result in a decreased phage titer. The scores for SEV on CQAs were rated at 1, and on PPA as 3.

The likelihood of failure is very low (OCC = 1), as the phage inoculum volume is measured using graduated pipettes, ensuring accurate and consistent addition. DET was rated at 3 as there is no alarm to detect failure.

For the **optical density (OD) at phage addition**, deviations from the target value are unlikely to affect the CQAs. However, if the OD value at phage addition is too low, the multiplicity of infection (MOI) increases, which may shorten the phage production process step since all the susceptible bacteria will quickly be killed. Conversely, if the OD value at phage addition is too high, the MOI decreases, potentially extending the phage production process step. The scores for SEV on CQAs were rated at 1, and on PPA as 3.

The likelihood of error is very low (OCC = 1), as the absorbance is regularly monitored and recorded. DET was rated as 1 since the OD is checked and documented regularly.

For the **multiplicity of infection (MOI)**, which is defined as the ratio of phages to host cell bacteria, deviations from the target value are not expected to affect the CQAs. When MOI is outside the expected range, it may influence the duration of the production culture and potentially affect the phage titer. If the MOI is below the target value, the ratio of host cells to bacteriophages will be higher, which could extend culture times until host cell lysis occurs. Conversely, if the MOI exceeds the target value, accelerated host cell death may occur, leading to a shorter latency phase and potentially resulting in a slightly lower phage titer. The scores for SEV on CQAs were rated at 1, and on PPA as 3.

The likelihood of error is very low and was rated as 1. The volume of phage inoculum suspension is measured with a serological graduated pipette to ensure accurate and consistent measurements, while bacterial cell concentration is monitored through OD.

DET was rated 3 since the obtained MOI is deduced from the phage titer in the phage inoculum and the bacterial cell concentration at phage addition and not directly measured.

The scores reported in Table 4 allow the easy identification of the criticality of PP for the phage amplification step. The pH,

Table 4: FMEA including SEV, OCC and DET scores for process variables of the phage amplification step.

	SEV CQAs								SEV PPA					
Process variable	**Identity**	**Bioburden**	**Endotoxin**	**Host-Cell Proteins**	**Host-Cell DNA**	**Temperate phages**	**Appearance**	**pH**	**Potency (Phage titer)**	**OCC**	**DET**	**CQA RPN**	**PPA RPN**	**Criticality**
Volume - Preculture	1	1	1	1	1	1	1	1	1	1	3	3	3	GPP
Volume - Medium	1	1	1	1	1	1	1	1	1	1	3	3	3	GPP
pH	1	1	1	1	1	1	1	1	3	1	1	1	3	**KPP**
Temperature	1	1	1	1	1	1	1	1	3	1	1	1	3	**KPP**
Stirring speed	1	1	1	1	1	1	1	1	3	1	1	1	3	**KPP**
Antifoam concentration	1	1	1	1	1	1	1	1	4	1	3	3	12	**KPP**
Volume - Phage Inoculum	1	1	1	1	1	1	1	1	2	1	3	3	6	GPP
OD at phage addition	1	1	1	1	1	1	1	1	3	1	1	1	3	**KPP**
MOI	1	1	1	1	1	1	1	1	3	1	3	3	9	**KPP**

temperature, stirring speed, antifoam concentration, OD at phage addition and MOI were identified as KPPs. These parameters will be characterized experimentally in a defined design space during Process Characterization (PC) studies for more insight of their influence on phage titer at this process step.

The same risk assessment was performed for all the other process steps in order to characterize the full production and purification process for *P. aeruginosa* phages.

4. Conclusions and Outlook

A FMEA risk assessment was conducted within the QbD framework to proactively identify risks associated with a *P. aeruginosa* phage production process. By assessing the criticality of PPs and MAs, this approach supports the establishment of a well-characterized and controlled manufacturing process. Ultimately, it lays the groundwork for improved consistency, scalability, and regulatory compliance, while ensuring high-quality phage products suitable for therapeutic applications.

Acknowledgements

We thank Nathalie Bonvin, Aurélie Marchet, Sabrina Pereira-Pipa, and Norah Alibrando for excellent technical assistance.

Author Contributions

C. J.: Conceptualization (lead), writing - original draft (lead), writing – review and editing (equal); S. G.: Conceptualization, writing – original draft, writing – review and editing (equal), J. F. B.: Conceptualization writing – review and editing (equal); G. R.: Conceptualization, writing – review and editing (equal).

Received: May 16, 2025

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