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White Paper

Aseptic Process Sampling Risk Mitigation – A Regulatory Perspective

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There are significant consequences associated with microbial contamination during biopharmaceutical manufacturing. Contamination increases risks for the operator, the company, and potentially the patient, all of which can result in significant negative impact. The contamination of a biologic can result in a lengthy shut down of a facility to conduct the investigations necessary to identify the root cause and prevent reoccurrence and worse, delay production and delivery of critical, life-saving medicines.

Sampling of biopharmaceutical process intermediates and the final product is essential for manufacturing workflows where the final product cannot be terminally sterilized. In addition to ensuring patient safety through bioburden monitoring, sampling is needed to support several key imperatives as driven by global regulatory trends including process analytical technologies (PAT) and quality by design (QbD) (Figure 1).

Among the drivers for improved, more robust sample processing is the need to assess the state of the process in terms of verifying, detecting, and adjusting the parameters required to produce the drug product. A second driver is the need to extract materials for later assessment. The materials extracted must be representative and in a quantity that is adequate for regulatory requirements in terms of sample storage for later assessment. Finally, sampling is needed for the transfer of materials, including adjustment by addition of different raw materials or seeding by inoculation.

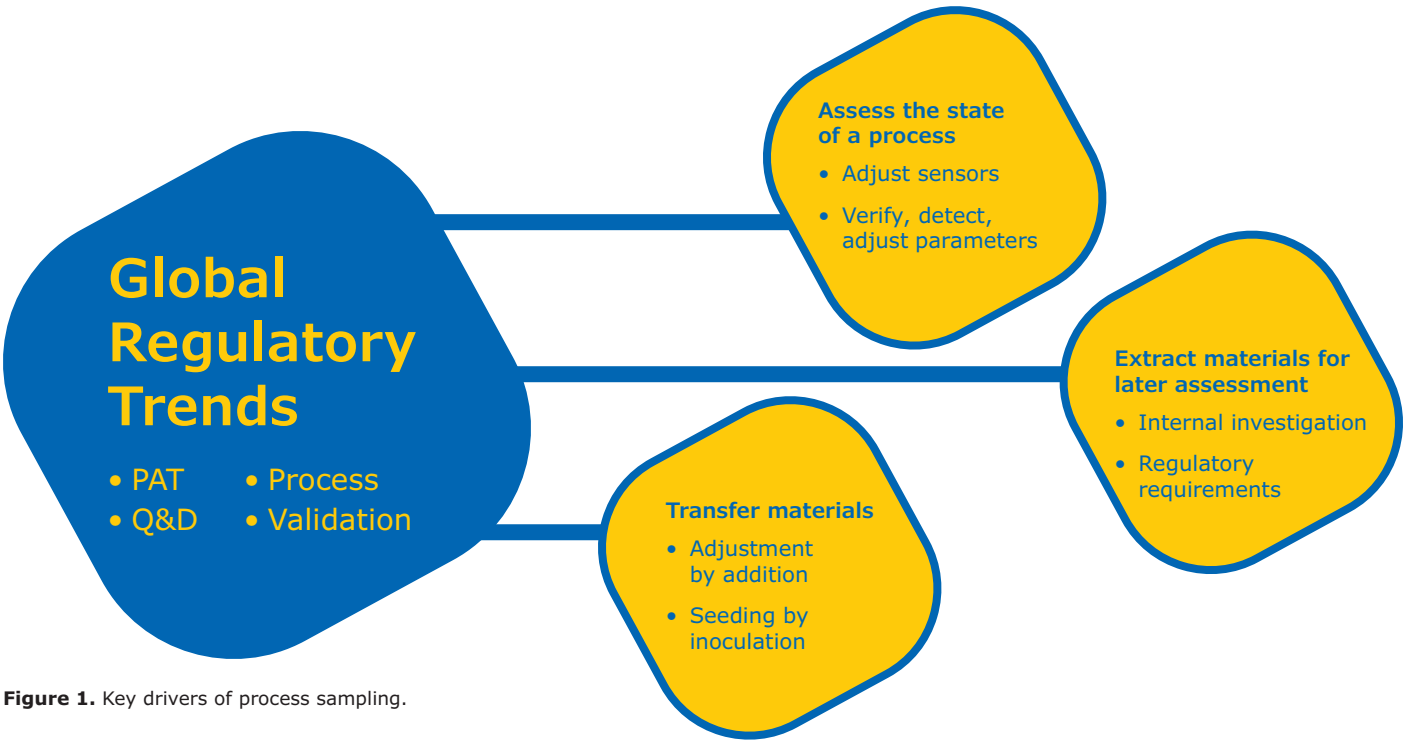

Figure 1. Key drivers of process sampling.

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Unfortunately, “traditional” sampling methods are not closed systems and therefore do not maintain a barrier to contamination entering the process during sampling. As a result, the sampling process itself, which is critical to the success and safety of the manufacturing workflow, can lead to contamination of a unit operation and possibly an entire batch. In contrast, aseptic sampling systems are disposable, closed units that always maintain aseptic conditions and ensure the security of the process, operator, and the sample.

This whitepaper provides a summary of regulatory guidelines for sampling, describes the traditional methods available for sampling and their limitations, and explores the advantages of aseptic, single-use sampling and how this approach more effectively aligns with regulatory guidelines.

Regulatory Guidelines for Sampling

In general, regulatory guidelines for sampling seek to protect the process, the sample and the operator and ensure a consistent method that is independent of the operator.

Table 1 provides an overview of regulatory recommendations and citations related to sampling. According to EU GMP Annex 1 revision March 2009, the bioburden should be monitored before sterilization and there should be working limits on contamination immediately before sterilization, which are related to the efficiency of the method to be used.[1] For regulators, this ensures that the bioburden requirements are met before sterilization in terms of verifying a sterility assurance level and a final bioburden level. Where overkill sterilization parameters are set for terminally sterilized products, the bioburden might be monitored only at suitable scheduled intervals. For aseptic processing, however, the bioburden must be monitored before sterilization on every batch.

The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S) has an interpretation for filtration specifically, stating that the filter efficacy studies must be considered when determining the acceptance criteria for the bioburden prior to filtration. This means that when two subsequent filtration steps are used, the product must be sampled prior to the last filtration step, if technically possible. If one fails the required sterility assurance level, then the manufacturer must identify ways to mitigate that risk. Moreover, knowledge and trending of the bioburden is critical from a regulatory perspective in terms of process control.

Annex 4 of the WHO guidelines covers the process for sampling of pharmaceutical products and related materials.[2] According to section 2.1 (preparation for sampling), all sampling tools and implements should be made of inert materials so that they do not interact, or react, with the sample itself, thereby affecting the analytical results. They should be stored in clean conditions and thoroughly washed and rinsed, before and after use. The section also states that the use of disposable, or single use, sampling materials has distinct advantages.

Section 2.2 (sampling operations and precautions) highlights the need for written procedures to be in place to describe the sampling operation. These procedures should include details of the health and safety of the operator during sampling. They should also ensure that representative samples are taken in sufficient quantity for testing at that time, during the process, and for storage for testing at a later time. Section 2.3 (storage and retention) states that the

Regulatory Recommendations	Citations
Contamination Control **AND** **Monitoring for every step before Bioburden Reduction**	**PICS/S – FDA cGMP –** Part I §5.19 -f Use of closed system recommended from phase 1 **WHO Annex 4** "The use of disposable sampling materials has distinct advantages" **WHO Annex 2 – ICH Q7A –** GMP guidance for API **EU GMP Annex 1** **EudraLex** – Vol 4 – PArt II – 2009
Operator Bias Elimination	**WHO Annex 4**
Representative Sample	**FDA**
Health and Safety Focus	**European Pharmacopeia** Guidelines for Sampling of Pharmaceutical Products and Related Materials
Retained Samples	**FDA CGMP** Guidance for the industry investigational drugs section F. Laboratory Controls / 1. Testing 2 years after expiration date / completion of trial and twice the quantity necessary to perform all tests

Table 1. Summary of regulatory guidance for sampling.

container used to store a sample should not interact with the sampled material nor allow any contamination. Moreover, the container should be sealed and preferably have a tamper-evident system in place.

FDA cGMP guidelines for Phase I drugs recommends the use of closed systems to minimize the risk of contamination.[3] These guidelines also recommend that the sample consist of appropriate, or adequate, quantities to perform additional testing if required, at a later date. Sometimes the stability of the sample or the post-process lot verification is out of specification, and so it may be necessary to test certain samples that are stored. These samples should be retained for at least two years.

To control contamination during sampling, the ICH Q7A Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients, which has also been adopted by the regulatory bodies of the European Union, Japan and the United States, states that the procedures used for in-process sampling should be designed to prevent contamination and ensure the integrity of samples after collection.[4] The WHO and European GMP guidelines also follow the ICH guidance in terms of in-process sampling and controls, highlighting the importance – and harmonization – around the globe to prevent contamination during sampling. In their guidance on sampling of pharmaceutical products and related materials, the WHO highlights the importance of training personnel carrying out the sampling procedure in the proper techniques and procedures to be used.

Limitations and Risk of Traditional Sampling Methods

"Traditional" sampling methods are not closed systems which means they do not maintain a barrier to contamination of the process during the sampling process.

In recent years, the number of microbial contamination issues reported to the FDA has increased, specifically for sterile injectable drug products. The lengthy investigation of these cases identified many problems such as incorrectly fitted components, missing O rings, deformation of an air filter after sterilization, as well as problems with the sampling devices.[5] Immediate corrective actions were put in place to solve these issues, but long-term actions were also implemented in order to improve the reliability of the manufacturing processes.

While areas were identified for improvement (preventative maintenance plans for all fermenter valves, including valves on sampling devices, as well as documentation for correct assembly of components), non-optimized sampling processes and procedures remain a problem. In 2015 alone, more than 120 FDA 483 warning letters were issued; 104 were issued for procedures designed to prevent microbiological contamination of sterile drug products not established, written or followed and 24 for not obtaining representative samples.

Although traditional sampling methods offer some advantages to manufacturers, many of these methods come with significant limitations (Table 1).

Challenges and Limitations with Respect to Regulatory Requirements of Traditional Sampling Methods

Method	Pros	Cons	Limitations
Open Sampling Valve	• Ability to collect large number of samples • Low cost/sample	• Dead-leg • Requires pre-flush which results in loss of product • Open transfer of sample • Impossible to sterilize	• High risk of contamination • Operator and process safety • Is sample representative?
SIP/Valve	• Ability to collect large number of samples • Closed sampling; low risk of contamination • Low cost / sample • Chemical compatibility of glass bottles	• Complex operation, especially if not automated • Requires SIP and cooling between sampling events (~60 mins) • Safety hazard (hot, pressure) • Condensate may dilute sample • Requires vented glass bottles that need to be cleaned and stored • Sampling container limitations	• High risk of contamination • Operator and process safety • Is sample representative?
Septum Samplers	• Ability to collect large number of samples • Low cost / sample	• Not steam sterilizable • Safety hazard (needles) • Limited to small volume samples	• High risk of contamination • Operator and process safety • Is sample representative?
Aseptic Connectors	• Flexible • Reliable • Validated connection for the transfer of sterile fluids • Safe and disposable	• Added cost • Potential dead-leg with tubing • Limited options for disconnection	• Material loss in case of dead-leg • Operator training
Tube Welders	• Ability to collect a high number of samples • Good aseptic sampling on most recent welders • Flexibility	• Waste of product • Require utility, lack of mobility • Time consuming (~6-10 mins/weld) • Capital investment of hardware	• Require additional maintenance with training needs • Operator training

Table 2. Summary of the pros and cons related to traditional sampling methods

Open Sampling Valve

The advantages of an open sampling valve include low cost and the ability to collect a large number of samples. Design limitations with dead-leg, however, can require flushing between each sample, constituting a pure loss of product. Moreover, this method is an open operation and is impossible to sterilize. These limitations conflict with the majority of requirements from regulatory authorities.

Steam in Place Sample Valve

A steam in place valve offers the same advantages as an open sampling valve but with a lower risk of contamination. Due to the steam required between each sample, however, there are operator safety concerns with the use of these valves. Moreover, steam after cooling can induce condensate, which will dilute the sample and alter its representativeness, and the operation procedure is also more complex and less flexible in terms of container requirements (limited to vented glass bottles). Overall, operator safety and sample representativeness are the major limitations of this sampling method.

Septum Sampling

Septum sampling offers the same advantages as the two previous methods but is limited by safety concerns linked to the use of a needle and a high risk of contamination. Furthermore, this method is limited to only small volume samples.

Aseptic Connectors

Aseptic connectors offer a safe, disposable, and validated connection for the transfer of sterile fluids, and are usually accompanied with a significant validation package established by the vendor. Nonetheless, this method is an expensive solution with design constraints (dead-leg due to the tubing, and limited options for disconnection). Because of the potential dead-leg, the risk of material loss or lack of representativeness cannot be precluded. These connectors also require operator training.

Tube Welders

Tube welders offer a flexible solution (choice of containers), the ability to collect a high number of samples, as well as good aseptic sampling on most recent welders. However, the technology is subject to disadvantages including product loss, lack of mobility, and capital investment. The welding cycle is a time-consuming operation, which requires the use of a specific hardware, initially acquired and maintained with preventative maintenance plans. This solution also requires operator training.

Closed Sampling

Given the shortcomings of traditional sampling, it is not surprising that a large number of biopharmaceutical companies have adopted closed, single-use sampling technology. A closed design ensures the process sample will be isolated from point of sample to analysis, reducing the risk of losing valuable product while maintaining the integrity of the fluid samples.

As shown in Figure 2, closed, aseptic process sampling offers several advantages when compared to traditional methods, including ease of use, better alignment with regulatory requirements and a limited investment. The specific ways in which closed sampling meets regulatory recommendations are summarized in Table 3 and include contamination control, elimination of operator bias, the ability to collect representative samples and improved health and safety.

Figure 2. Sterile single-use processing offers key advantages over traditional methods.

Regulatory Recommendations	Citations	Corresponding Need	Aseptic Sampling Benefits
Contamination Control	**WHO Annex 4** – Guidelines for Sampling of Pharmaceutical Products and Related Materials **WHO Annex 2** – GMP for active pharmaceutical ingredients **ICH Q7A** – GMP guidance for API **EudraLex** – The Rules Governing Medicinal PRoducts in the European Union, Volume 4, EU Guidelines to GMP, Medicinal Products for Human and Veterinary Use, PArt II, Basic Requirements for Active Substances used as Starting Materials	A closed, pre-sterilized disposable system minimizes this risk dramatically, by eliminating contamination entry points and cleaning validation challenges. Citation from WHO Annex 4 regulatory guidelines: **"The use of disposable sampling materials has distinct advantages."**	YES
Operator Bias Elimination	**WHO Annex 4** – Guidelines for Sampling of Pharmaceutical Products and Related Materials	Simple procedure that eliminates complexity of training, reducing risk of operator bias	YES
Representative Sample Requirement		No steam condensate or dead leg flush that may dilute sample	YES
Health and Safety Focus		Elimination of steam sterilization (high heat/pressure) and glass bottle safety risks.	YES

Table 3. Sterile sampling addresses specific regulatory recommendations.

Facilitating Continuous Process Improvement

Continuing process improvement with sampling includes development, process validation and life cycle management, all of which are facilitated with the use of aseptic sampling. For development, it is important to efficiently analyze process intermediates and conditions so that additional time is not added to the process that could affect the results. It is also important to understand critical material attributes (CMA) and process parameters (CPP) that impact the final drug product quality attributes (CQA). During process validation, a design space is defined based on those critical parameters, analyzing the impact of time on materials, and then lining up any requirements needed based on regulatory requirements or standards. In terms of life cycle management, statistical sampling at key points is essential for process monitoring, trend monitoring, understanding optimal frequency, and where the representative samples need to be taken. There also needs to be a feedback loop to make real-time adjustments to maintain a state of control. These are all driven by modern process validation approaches, utilizing quality by design (QbD) and process analytical technology approaches.

Verification of Virus Inactivation: A Sterile Sampling Case Study

The value of closed sampling is exemplified by this case study describing the approach used to verify virus inactivation. A biopharmaceutical company that incorporated virus inactivation as a process step in cell culture production performed kinetics to verify the inactivation only during validation. However, the FDA recommends performing inactivation kinetics during validation and manufacturing. Virus inactivation requires up to 15 samples every five to ten minutes, which is not possible with traditional sampling using CIP/SIP valve and glass bottles. The solution, which was selected to achieve FDA expectations, was to implement an aseptic sampling solution based on closed 60 mL PETG bottles plus the set of suitable connectors needed to connect these units to the tank. In addition to meeting regulatory requirements, the benefits achieved by the company included a more rapid and accurate process and easier sample handling in the quality control (QC) Lab.

Summary

A biopharmaceutical manufacturing process cannot be controlled without the proper sampling. And while many options exist for sampling, not all are well-positioned to deliver the desired benefits or fully align with regulatory guidelines. Selecting a robust and optimized method along with implementation of an appropriate sampling plan results in compliance with regulations and better process and sampling management. Not surprisingly, the biopharmaceutical industry is increasingly recognizing the value of closed sampling and taking advantage of the many benefits to protect the process, operators and ultimately, patients.

NovaSeptum® GO Sterile Sampling

The unique technologies of the NovaSeptum® GO™ sterile sampling system results in faster turnaround time between samples while minimizing loss of product and the risk of contamination when compared to traditional methods such as SIP valve/welding (Table 4). Integrated security capabilities provide an extra level of confidence in risk mitigation strategy by allowing the device to be locked and containers sealed when not in use, keeping samples safe and the process under control.

Features	NovaSeptum® GO™ Single-use Sampling	Traditional Method SIP Valve/Welding
Time to first sample	Immediate	20 - 40 minutes
Time between samples	2 minutes	40 - 80 minutes
Safe for both the process and operator	YES	NO
Unbreakable containers	YES	NO
Multiple container configurations	YES	NO
Ease to sample	YES	NO
Adaptable within any process	YES	NO
Maintenance free	YES	NO
No dillution due to condensation	YES	NO
Compatible with single-use process	YES	NO

Table 4. Sterile sampling results in faster turnaround time between samples while minimizing loss of product and the risk of contamination.

References

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