SANISURE
Solution Based Innovation

Ensuring Nuclease-Free Process Consumables for Biopharma Applications

Richard Fry, PhD, SaniSure

Executive Summary

Advanced therapies, including cell and gene therapies and RNA-based drugs, represent a transformative shift in modern medicine. These therapies demand contamination-free manufacturing environments to ensure their safety, efficacy, and consistent quality. Even trace levels of contamination, particularly from nucleases such as DNase and RNase, can compromise critical materials like plasmid DNA and mRNA, undermining therapeutic outcomes.

As the demand for RNA-based vaccines and advanced therapies continues to grow, the industry faces increasing pressure to establish stringent contamination controls across all stages of production. Ensuring nuclease-free environments has become essential for maintaining the potency of sensitive therapies, highlighting the critical need for innovative manufacturing solutions.[1]

This article explores how innovative manufacturing processes, rigorous testing protocols, and quality by design (QbD) can yield nuclease-free, GMP-compliant process solutions, safeguarding sensitive biopharmaceutical materials.

The Nuclease Challenge

DNase and RNase enzymes present significant risks in biopharma processes, particularly for advanced therapies like plasmid DNA and mRNA therapeutics.[2] These nucleases degrade DNA and RNA molecules, potentially compromising the quality and efficacy of therapeutic products.[3] Even trace levels of DNase or RNase can disrupt synthesis processes, reduce yields, and render materials unsuitable for therapeutic use.

Storage in nuclease-free containers is critical for preserving the quality of plasmid and mRNA products.[4] Containers designed with advanced contamination controls and validated nuclease-free status provide a robust solution for storing and handling sensitive biopharmaceutical materials.

The U.S. FDA, EMA, and other major regulatory bodies establish GMP requirements with strict contamination controls to ensure consistent and contamination-free processes, which are essential for maintaining a nuclease-free environment. Achieving this level of control requires that process equipment and consumables start in a low- or nuclease-free state. A robust QbD approach to manufacturing a nuclease-free product involves eliminating nuclease contamination risks by creating controlled environments, eliminating human contact until the consumable is fully closed, and utilizing filtered air for process air requirements. Periodic verification testing is then conducted to certify nuclease-free status.

PharmaTainer™ Manufacturing Excellence

PharmaTainer™ bottles are manufactured using 100% virgin ADCF (animal-derived component-free) certified resins, ensuring safety and compatibility for biopharmaceutical applications. The manufacturing process is meticulously designed to eliminate contamination risks while maintaining the highest quality standards.

The injection stretch blow molding (ISBM) process used by SaniSure begins with the creation of an injection-molded preform. This preform features a fully formed neck closure thread and closed container base, ensuring a closed and controlled structure. Unlike extrusion blow molding (EBM), which forms a tube with an open thread and a base that is sealed during extrusion, the ISBM process preserves the integrity of the neck area. The preform is then conditioned to maintain the precision of the neck during the subsequent stretching and blow molding stages. This ensures that each PharmaTainer™ has a perfectly formed neck, allowing for airtight seals with double-seal, gasket-free caps. The closed, molded base also eliminates the potential for particle generation during the extrusion base closing process.

In contrast, competitors employing EBM face significant challenges, including variability in wall thickness, neck flash, and the need for gaskets. The cutting and closure step inherent to the EBM process at the base of the bottle introduces a risk of particle generation and structural inconsistencies.

These issues can compromise sterility and create handling risks, such as contamination from sharp burrs or weak seals.[5] Additionally, the EBM process generates more scrap material, reducing efficiency and sustainability compared with ISBM.

The ISBM process ensures consistent wall thickness and eliminates defects like neck flash, resulting in a burr-free finish. Contamination risks are minimized through the use of 0.2-micron filtered air during blow molding, with all operations conducted in ISO Class 5 cleanroom conditions up to the capping stage. The bottles are then vacuum sealed in their primary packaging to provide tamper evidence and sterility assurance at the point of use. To support seamless transitions into cleanroom environments, two additional layers of packaging are included, allowing for staged removal as the product moves through different zones. Final labeling, inspection, and secondary packaging are completed in ISO Class 7 conditions, ensuring clean inner packaging surfaces for introduction into clean room processing environments.

The table below illustrates that PharmaTainer™ bottles have the most robust characterization package in the industry.

PharmaTainer™'s ISBM manufacturing process embodies the principles of QbD, ensuring superior process control, product consistency, and contamination control. This robust approach supports the sterility validation process by providing the controlled and consistent manufacturing environment necessary for reliable sterility assurance. Sterility itself is ensured through a validated gamma or X-ray irradiation process conducted in accordance with ISO 11137 standards. Unlike competitor products, which are often produced in generic facilities designed for domestic plasticware, labware, or general single-use products, PharmaTainer™ is manufactured in a facility dedicated exclusively to producing sterile single-use consumables for the biotech and vaccine industries. Together, these measures deliver a dependable solution for storing and transporting sensitive materials in biopharma manufacturing, addressing customer demands for quality, safety, and efficiency.

DNase/RNase Testing and Validation

Initial risk assessments for the PharmaTainer™ manufacturing process identified no potential sources of DNase/RNase contamination. PharmaTainers™ are completely manufactured using robotics and capped in ISO 5 conditions before the first opportunity for contamination is identified. In order to confirm the absence of nuclease, PharmaTainers™ were tested for DNase/RNase.

Testing	Standard	Details	Frequency
Visual Inspection	N/A	Visual inspection for visible particulates and manufacturing defects.	100% in-process
Particulates	USP <788>	Automated test via light obscuration. Typical results <2% of USP limits for large-volume parenteral @ 10 and 25 µm.	Routine monitoring, 2× per shift
Endotoxin	USP <85>	5% rinse volume with 0.001 EU/mL limit.	Lot release
Bioburden & Sterility	ISO 11137	SAL 10-6 based on ISO 11137. Includes process validation, quarterly dose audits, and bioburden assessment.	Quarterly
Nucleases	Commercially available RNase detection assay	Risk assessment: No inherent risk of nuclease contamination. Testing confirms RNase free.	Product qualification, quarterly routine monitoring
Extractables	USP <665> "High Risk"	50% ethanol, acid, base, & USP water at 21 & 70 days.	Product qualification
Biocompatibility	USP <87>, ISO 10993-05, USP<88>, ISO 10993-06, -10, -11	Includes *in vitro* cytotoxicity, *in vivo* muscle implantation, intracutaneous, and systemic toxicity.	Product qualification
Freezing	In-house drop and gas leak test methods	Water-filled PC & PET PharmaTainer™, frozen to –80 °C dropped from ≥1M; thermal cycling from +25 °C to –80 °C.	Product qualification
Plastic Containers	USP <661>	Pass: NVR, residue on ignition, heavy metals, and buffering capacity.	Product qualification

PharmaTainer™ bottles underwent rigorous DNase and RNase testing to validate their nuclease-free status, addressing the critical need for contamination control in biopharma manufacturing.[6] Testing protocols ensure the products meet stringent customer requirements, safeguarding sensitive materials like plasmid DNA and mRNA during storage and transport.

The testing process was conducted in collaboration with a leading nuclease testing company, using validated sampling methods and highly sensitive detection assays. This collaboration underscores SaniSure's commitment to leveraging expert partnerships to ensure the highest standards of quality. The risk-based approach used during validation identified and mitigated potential contamination sources, ensuring reliable results across multiple production batches.

Quarterly routine monitoring is now in place to maintain this high standard, with the flexibility to increase testing frequency based on customer demand. These stringent measures demonstrated consistent negative results using an assay for the presence of DNase or RNase contamination and validating PharmaTainer™'s ability to provide nuclease-free environments for advanced therapies. By combining advanced manufacturing processes and controls, meticulous validation protocols, and proactive risk assessment, SaniSure delivers confidence and reliability for customers developing lifesaving therapies.

Regulatory Compliance and Standards

PharmaTainer™ bottles help biomanufacturers establish and maintain a state of control over potential contaminants, ensuring they are suitable for the most critical biopharma applications. The containers are designed and manufactured in compliance with key global regulations, providing customers with confidence in their safety, sterility, and reliability.

Regulatory Frameworks

PharmaTainer™ bottles are designed and manufactured to support compliance with FDA 21 CFR Part 211.65, which outlines requirements for equipment used to prevent contamination, and FDA 21 CFR Part 820, which provides quality system regulations for medical devices. While PharmaTainers™ themselves are not regulated products, the manufacturing processes align with these standards to ensure that biopharmaceutical manufacturers can confidently integrate the bottles into GMP-compliant workflows. Additionally, the bottles are manufactured in facilities and under conditions that meet the principles of EMA Annex 1 (EudraLex Volume 4), which emphasizes the importance of contamination control in sterile pharmaceutical manufacturing environments.

Third-Party Validation

PharmaTainer™ undergoes rigorous testing by specialized third-party laboratories to support its biocompatibility, extractables, sterility, and DNase- and RNase-free claims. These validations confirm the absence of nucleases and demonstrate the integrity of manufacturing processes and environmental controls.

In-House Testing

PharmaTainer™ undergoes rigorous testing for more frequently tested attributes to support its endotoxin and particulates claims. This routine monitoring confirms the continuous control of the manufacturing process.

PharmaTainer™ enables biopharmaceutical manufacturers to reliably maintain contamination-free solutions, meeting the needs of the most sensitive applications. This robust QbD manufacturing process underscores the product's value for critical biopharma applications.

Differentiating PharmaTainer in GMP and Scale

While many containers on the market claim to be nuclease-free, the vast majority are designed for laboratory settings and small-scale applications, such as pipette tips or containers/bottles up to a maximum volume of 2 L. These products often lack the rigorous qualifications required for GMP environments, making them unsuitable for large-scale biopharma manufacturing.

PharmaTainer™ bottles, in contrast, are engineered, manufactured, and controlled specifically to meet the stringent demands of GMP processes at scales from 10 mL up to 20 L. This scalability is critical for applications, such as mRNA vaccines and gene therapies, where consistent performance and sterility are essential across production volumes. Unlike labware manufacturers, SaniSure provides validated GMP solutions for the full range of manufacturing needs, ensuring compatibility, sterility, and compliance at every step.

By bridging the gap between laboratory claims and true GMP-compliant solutions, PharmaTainer™ sets a new standard in reliability and performance for biopharma manufacturing.

Conclusion

PharmaTainer™ bottles, a hallmark of SaniSure's innovation, redefine contamination-free storage for critical biopharma applications. By combining advanced ISBM technology, rigorous DNase/RNase validation, and the most comprehensive validation guide in the industry, PharmaTainer™ offers unmatched reliability for GMP-compliant storage and transport. Furthermore, PharmaTainer™ bridges the divide between laboratory innovations and GMP manufacturing requirements, offering unmatched scalability and reliability.

SaniSure's dedication to addressing evolving industry needs is evident in PharmaTainer's™ design and production. From ensuring sterility and scalability to providing robust solutions for mRNA and advanced therapies, SaniSure empowers manufacturers to protect sensitive materials and optimize workflows. As the demand for cutting-edge bioprocess solutions grows, SaniSure remains at the forefront, offering unparalleled quality, innovation, and support for life-saving therapies.

1230 Calle Suerte Camarillo, CA 93012, USA
P: +1 800 726-4835 E: SalesSupport-US@sanisure.com

References

1. Dezolt, F., Camilo, C., & de Souza, A. P. (2024). "Nucleic acid degradation as barrier to gene delivery: A guide to understand and overcome nuclease activity." *Chemical Society Reviews*.
2. Seifert, David. (2024). "RNase Contamination — Challenges & Solutions." Single Use Support.
3. Van Pottelberge, Robbe, et al., (2024). "Importance of RNase monitoring during large-scale manufacturing and analysis of mRNA-LNP based vaccines." *Journal of Pharmaceutical Sciences*.
4. Blom, H. (2022). "Reducing the risk of RNAse contamination in mRNA production." Cytiva.
5. Teknor Apex Company. (n.d.). Extrusion blow molding troubleshooting guide.
6. Henry E. et al. "Implementing routine monitoring for nuclease contamination of equipment and consumables into the quality Management system of a laboratory." Heliyon. 10: e24603 (2024).

About SaniSure

SaniSure is a global leader in developing and manufacturing single-use solutions for the bioprocessing industry. Our comprehensive product line includes high-quality components and assemblies, such as Mixed4Sure mixing systems, Cap2V8® Closure Systems, PharmaTainer™ Bottle Solutions, Fill4Sure Filling Assemblies, tubing, filling needles, bag systems, and other sanitary components, all designed to meet the stringent demands of sterile manufacturing.

With five production sites worldwide, our core manufacturing capabilities encompass injection molding, cleanroom assembly, tubing extrusion, injection-stretch blow molding, fabrication, and other proprietary technologies. This extensive expertise enables us to deliver innovative solutions tailored to each stage of the biomanufacturing process.

At SaniSure, our rigorous validation and continuous improvement processes reflect our unwavering commitment to quality and reliability. We deliver containers that biopharma manufacturers can trust for their most critical applications, ensuring the highest level of confidence for customers developing life-saving therapies. Our quality management system is certified to the ISO 9001:2015 standard, and we apply cGMP guidelines throughout our manufacturing operations. Our routine environmental monitoring program ensures our assemblies are manufactured in a clean and controlled environment, meeting the acceptance criteria set forth by applicable ISO and USP standards.

By integrating outstanding products with groundbreaking engineering, SaniSure is dedicated to advancing the biopharmaceutical industry through robust design, innovative manufacturing, and unwavering quality assurance.