



Filter Integrity Testing in Biopharmaceuticals: A Comprehensive Review of Methods, Challenges, and Future Directions

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Abstract:

Filter Integrity Testing (FIT) serves as a cornerstone of sterility assurance in pharmaceutical manufacturing, validating sterilizing-grade filters' performance throughout their lifecycle. This article examines FIT methodologies, equipment selection, and implementation challenges specific to biopharmaceuticals and advanced therapeutics. The article explores critical timing considerations for testing, non-destructive verification methods, and the complexities of closed-system cell therapy manufacturing. It addresses regulatory interpretation disparities while highlighting industry harmonization initiatives aimed at standardizing practices. The emergence of digital quality systems, computational modeling, and artificial intelligence applications demonstrates the evolution of FIT toward more predictive, data-driven approaches. Through critical analysis of technical limitations and case studies, this article provides a framework for implementing robust integrity testing strategies that balance regulatory compliance, operational efficiency, and patient safety in increasingly complex therapeutic modalities.

1. Introduction and Regulatory Framework

Filter Integrity Testing (FIT) represents a cornerstone of quality assurance in biopharmaceutical manufacturing, serving as a critical control point to verify that sterilizing-grade filters maintain their barrier function throughout processing [1]. This verification is essential not merely as a regulatory checkbox but as a fundamental safeguard ensuring product sterility, patient safety, and manufacturing reliability. As the biopharmaceutical landscape evolves toward increasingly complex modalities, including cell and gene therapies, the importance of robust FIT strategies has grown exponentially. The strategic significance of FIT extends beyond regulatory compliance to form a central element of contamination control and sterility assurance. In cell therapy manufacturing—where each batch may represent a single patient's treatment opportunity—filter failures can lead to catastrophic outcomes, including product contamination, patient safety risks, and substantial financial losses [2]. Studies have documented that a significant portion of sterility failures in aseptically filled products can be traced to compromised filter integrity, underscoring

the critical importance of robust testing protocols [1]. Filter integrity testing serves multiple purposes within the manufacturing workflow, including verification of proper filter installation, confirmation of filter integrity post-sterilization, validation of filter performance during processing, and documentation of system closure throughout manufacturing [1]. The timing of these tests—whether Pre-Use, Pre-Use Post-Sterilization Integrity Testing (PUPSIT), or Post-Use—directly impacts the effectiveness of contamination control strategies and the reliability of sterility assurance. Biopharmaceutical manufacturing employs diverse filtration objectives that necessitate specialized approaches to integrity testing. These objectives include clarification to remove cells and particulates from harvested cell culture fluid; bioburden reduction to decrease microbial contamination before or to sterile filtration; sterile filtration using 0.2 µm filters as the final barrier before filling; viral filtration with nanofiltration membranes (15-50 nm) to remove viral contaminants; and ultrafiltration/diafiltration for product concentration and buffer exchange [2]. Each filtration step requires tailored integrity testing strategies based on filter type, pore size, and

application criticality. As manufacturing platforms evolve toward closed systems, single-use technologies, and continuous processing, the implementation of FIT must adapt accordingly. The selection of appropriate testing methods and timing must balance technical reliability, operational feasibility, and regulatory expectations while supporting efficient manufacturing of safe and effective therapies [2].

2. Filter Integrity Testing: Technology and Equipment.

Filter integrity testing schedule is a crucial aspect of biopharmaceutical manufacturing regarding the quality of products and assurance of sterility. Pre-use testing, which is performed before exposure of the filter to process fluids, is used to establish the baseline of the subsequent test by confirming initial integrity and correct installation. Research has indicated that a significant proportion of filter failures are observed at the Pre-Use testing stage and, therefore, potential incidences of contamination are prevented before the commencement of the process [3]. Pre-Use Post-Sterilization Integrity Testing (PUPSIT), which is required by EU GMP Annex 1, is used to confirm the integrity of a filter following sterilization procedures like autoclaving or gamma irradiation, which can cause mechanical stress or material degradation. According to industry data, filters passing Pre-Use testing, but failing PUPSIT, commonly have microscopic defects that occurred during sterilization, with failure rates ranging between 0.5 and 2.8 factors of sterilization method and filter composition [3]. Post-use testing, which is done after filtration of the product, ensures that the filter has not altered during processing, and detection rates of failures due to processes are found to range between 1.2 and 3.7 in different applications. The timing strategies have their own purposes in the general scheme of the contamination control, and their joint application ensures a full guarantee of the filters functioning during the whole manufacturing cycle [3]. Non-destructive integrity testing is a powerful method of reliably verifying the performance of filters with no harm to the functioning of the filters. The Bubble Point Test is applied to hydrophobic filters and is used to measure the pressure at which the water continuously bubbles through a wet membrane, and its sensitivity can be used to detect defects as small as 2-3 micrometers based on the membrane composition. This is the highest pressure test among all the common tests, but it gives definitive visual confirmation of integrity breaches [4]. Diffusion Test, which is suitable in hydrophilic

filters, is used to measure the speed of gas diffusion through a moist membrane under a pressure lower than the bubble point, where defects that may otherwise escape the bubble point test are detected with a precision sufficiently sensitive to detect pinholes smaller than 1 micrometer in diameter. Comparison of detection capabilities. Studies have demonstrated that automated diffusion techniques can detect much smaller defects than can be detected using bubble point methods [4]. The Pressure Hold Test (a form of diffusion testing) is used to check the pressure drop across time on the upstream side of a wet filter to detect possible leaks, especially useful for the filters with a low flow rate or in cases where automated systems are not available. The Water Intrusion Test (WIT), created specifically to test the hydrophobic filters in use in gas filtration or venting systems, measures the pressure under which the water will intrude to test the integrity of the membrane without wetting the filter, and is sensitive enough to detect defects that may affect sterility in critical applications [4]. Filter integrity testing equipment selection includes a variety of technologies that are more or less automated, sensitive, and regulation-compliant. GMP-wise manufacturing settings are dominated by automated integrity testers, including Sartochek 5 Plus, Palltronic Flowstar, and Millipore Integritest 5, which provide standardized testing and data capture protocols, with electronic data capture and connection to manufacturing execution systems. Such systems test several types of tests (Bubble Point, Diffusion, Pressure Hold) that are highly sensitive and reproducible, minimizing the variability in operators that has been cited as a key cause of test inconsistency in comparative studies [3]. Manual pressure decay systems are still widely used in development laboratories because of their affordability and flexibility, but have a greater range of variability and do not capture automated data as needed in a regulatory context. Comparison of automated and manual systems has shown that there is a higher inter-operator variance in the use of a manual system than the automated platform [3]. In-line or at-line testing with minimum process interruption. Custom skid-based systems installed on single-use manufacturing lines are especially useful in discrete processes with a large volume and limited filter access, such as high-closed-loop processes. Although high in complexity and less flexible than independent testers, these fully integrated systems facilitate the continuous manufacturing paradigms through real-time integrity checks [3]. Filter properties greatly determine the testing methodology and reliability of the test of integrity (type (hydrophilic vs. hydrophobic), pore size, and material composition)

all determine the type of test to be used. Liquid-based sterilizing grade filters usually have $\mu\text{m}22$ pore sizes, whereas gas filtration utilizes $\mu\text{m}45$ or larger membranes, and their integrity test parameters are usually tuned to those two specific sizes [4]. Hydrophilic filters of polyethersulfone (PES) or cellulose acetate are tested by using automated testers since this has been proven to identify the small bubbles on wet membranes. The polytetrafluoroethylene (PTFE) hydrophobic filters, on the other hand, need Water Intrusion or Pressure Hold tests because they are not readily wettable by aqueous solutions. Comparison of the performance of integrity tests of different types of filters has resulted in the determination that bubble point values are significantly higher with hydrophilic PES membranes when compared with other types of pore-size cellulose acetate filters, requiring material-specific test parameters [4]. Virus filters that have a pore size between 15 and 50 nm have their own special problems in integrity checking, and Diffusion tests are better at finding defects at the nanometer scale. Such considerations related to the filters spotlight the criticality of validated and customized testing methods instead of generic testing methods, which could be ineffective in detecting material-specific failure modes [4]. Technical aspects of test reliability are wetting conditions, equipment calibration, operator technique, and environmental conditions. Poor wetting is one of the most frequent causes of false positives in bubble point and diffusion, and literature indicates that most apparent filter failures can be connected to poor wetting and not actual integrity failures [3]. Calibration drift in equipment can influence the accuracy of the measurements, and in fact, it has been reported that untested integrity testers may yield false values in many tests, especially those that involve important measurements that are close to specification limits. There is a lot of variance brought about by operator tech, especially in manual systems. When the procedures are not consistent, the test results of different technicians may vary greatly [3]. Environmental conditions also have an influence on the diffusion rates and pressure levels of the gas, and literature evidence shows that a difference of 5°C in temperature can change the outcomes of diffusion tests significantly, either leading to false positives or concealing the presence of a defect. All these technical considerations put forward the importance of detailed validation procedures and procedures that incorporate real-world variables as opposed to idealized lab conditions that are not necessarily relevant to manufacturing [3].

3. The difficulties surrounding the implementation of Cell Therapy.

Filter Integrity Testing (FIT) presents unusual challenges in the implementation in cell therapy manufacturing due to closed-system process considerations and strict time constraints. In contrast to conventional biologics, cell therapy products frequently utilize single-use and closed system designs to reduce risks of contamination, and it was shown that such closed systems are capable of controlling the occurrence of microbial contamination, relative to open processing [5]. Nevertheless, such closed systems also severely hinder access to filters at their usage, constraining the possibility of an overall integrity check during the manufacturing process. A survey among the cell therapy manufacturers has indicated that most companies are faced with a major challenge of conducting Post-Use FIT because of constraints of closed systems, which is forcing them to rely more on Pre-Use and Pre-Use Post-Sterilization Integrity Testing (PUPSIT) [5]. Timing sensitivity also creates further problems with many cell therapy products having such short shelf lives of hours, not days, leaving tight timeframes to perform testing procedures. This limitation is especially acute when using the cryopreserved intermediates that impose complicated freeze-thaw cycles that can influence the integrity of the membranes, and there have been reported cases of filter failure rates escalating after cryogenic exposure. According to industry reports, many cell therapy manufacturing companies have reported a break in schedules due to challenges in timing of FIT, which highlights the necessity of a fast, simplified testing process that does not jeopardize manufacturing schedules [5]. The fact that the technical limitations and variability of the method considerably affect the reliability of the FIT results in cell therapy applications is important. Traditional techniques, such as bubble point and pressure hold test, though largely used, might be insensitive to the identification of micro-defects or pinholes in filters utilized in important cell therapy procedures. It has been shown in comparative studies that standard bubble point testing is unable to detect defects smaller than a few micrometers, and may thus permit the entry of contaminants past filtration barriers in tested filters [6]. Variability of methods adds further uncertainty, and interlaboratory studies have found a range of variation in results of the same filters under allegedly standardized conditions. This variability is worsened in a cell therapy setting, with batch sizes being small and the margin of error being very low, with some centers reporting large percentages of false positives with particular filter

configurations [6]. Subjectivity in test results interpretation: Interpretation of test results manually introduces subject, especially where borderline measurements arise at the boundaries of the specification, resulting in inconsistent decision-making. In addition, legacy equipment without digital data capture functions makes trending analysis and deviation investigation more difficult, and surveys show that, at this point, many cell therapy facilities continue to use somewhat manual equipment with limited data integration potential. All of these technical shortcomings undermine the trustworthiness of the results of the FIT and force the use of redundant testing plans that require the use of valuable time and resources in what are already limited manufacturing processes [6]. Equipment qualification and operator training are major pitfalls in the implementation of the FIT in the cell therapy platforms. Human error factors like imprecise test settings, manual handling errors, and deviation from procedure are significant factors that can cause variability in test results. Recent surveys of the industry have shown some terrible statistics: a large percentage of fits deviations could be attributed to operator technique and not to a real filter failure, and in most cases, inadequate training can be noted as the root cause [5]. This is exacerbated by the specialized character of cell therapy production, with technicians typically not possessing large volumes of experience with filtration systems because of the usual situation with the traditional operators of biopharmaceuticals. The qualification practices of equipment often have a gap, especially in Performance Qualification (PQ), which confirms the performance of the system in real operating conditions. Although this is usually done in a majority of the facilities, as demonstrated by evaluations by industries, several do not have strong PQ protocols dedicated to cell therapy applications [5]. This is particularly worrying given that filters used in cell therapy manufacturing frequently face distinct operating environments, such as variable viscosities and particle loads, and operating temperatures, which might not be well modeled in typical qualification procedures. This poor operator training, coupled with insufficient equipment qualification, has produced an ideal tempest of FIT failures and collateral impacts on manufacturing reliability, batch release schedules, and, eventually, access to life-saving treatments by patients [5]. Filter-specific validation methods pose significant difficulties in the production of cell therapies, with filter types and materials, and configurations being varied, requiring specific integrity testing methods. Filter sterilization needs to be checked under design-specific, pore-size-

specific, and application-specific validated methods, but industry surveys have found that most facilities use generic or unvalidated methods of checking that do not effectively identify breaches in particular filter design configurations [6]. This risk is further increased with special filters in cell therapy processing, including filters in cryopreserved product filtration or filters in complex single-use assembly. Filter properties such as pore size distribution, material composition, and mechanical properties have a direct effect on test sensitivity and reliability, and research has shown that changes in these properties can result in test result variability when inappropriate methodologies are used [6]. This complexity with materials further makes it valid, effectively, because advanced filter designs with multiple layers, composite materials, or novel polymers can behave unpredictably when put through commonly used tests of integrity. Studies have revealed that even though multi-layer filters may contain defects in inner layers, which give a false impression of security, they can pass bubble point testing. These filter-specific factors lead to the need to have extensive validation plans that consider material characteristics and process states and failure modes that apply to cell therapy applications and not general schemes that were developed with regard to traditional biologics [6]. An interesting example of a commercial CAR-T production facility describes how high the stakes are in the case of cell therapy failures. A post-use filter integrity test during a patient-specific production run failed after a successful pre-use test and PUPSIT test, leading to batch rejection and beginning a complicated root cause investigation. It was found that a combination of factors led to such an outcome: the wetting of the hydrophilic filter was not done properly during the first setup, and the calibration drift of the automated integrity tester, which had not been identified in several months [5]. These technical problems, exacerbated by poor operator training in filter handling processes unique to cell therapy applications, cost a treatment opportunity to a critically ill patient in need of cellular therapy. The manufacturing delay resulted in the rearrangement of the conditioning regimen and drawing of new starting material for the patient and prolonged treatment schedules by a few weeks [5]. Monetary costs also included R&D, direct manufacturing losses, and other costs linked to investigatory resources, remedial measures, and prophylaxis in the entire network of manufacturing operations of the organization. This example illustrates the chain of events that have resulted because of the failure of FIT in cell therapy situations, where each lot is generally a single treatment experience, and sterility guarantee is

desperately significant to the well-being of the patient and the economic feasibility of the business. The changes put in place, such as the increase in operator training, equipment requalification, and process-specific validation protocols, illustrate the multifaceted nature of the response that was needed to combat the challenges of FIT in advanced therapy manufacturing [5].

4. Standardization and Innovation

Regulatory interpretation disparities present significant challenges in the implementation of Filter Integrity Testing (FIT) across biopharmaceutical manufacturing networks. While regulatory frameworks, including FDA's 21 CFR Part 211, EU GMP Annex 1, and USP, <797> all mandate filter integrity verification, their specific requirements and implementation expectations vary considerably, creating ambiguity for global manufacturers [7]. The EU GMP Annex 1 explicitly requires Pre-Use Post-Sterilization Integrity Testing (PUPSIT) for all sterilizing filters, while the FDA has historically adopted a more risk-based approach, allowing manufacturers to justify alternative strategies based on product criticality and contamination risk [7]. These divergent interpretations create operational complexities for multinational organizations, with industry surveys revealing that many manufacturers maintain distinct testing protocols for products destined for different markets. The implications extend beyond procedural differences to impact equipment selection, documentation requirements, and validation strategies. Adding further complexity, regulatory guidance documents such as the FDA's "Sterile Drug Products Produced by Aseptic Processing" and EMA's "Manufacture of Sterile Medicinal Products" offer differing perspectives on testing methodologies, acceptance criteria, and failure investigations [7]. Contract development and manufacturing organizations (CDMOs) serving multiple sponsors face particular challenges, as they must navigate diverse client expectations while maintaining consistent quality systems. These disparities not only increase operational complexity but also create potential compliance vulnerabilities during regulatory inspections, with documented instances of facilities receiving contradictory observations from different regulatory bodies regarding identical FIT approaches [7]. Industry harmonization initiatives seek to establish standardized approaches to FIT that satisfy global regulatory expectations while enabling operational efficiency and technical reliability. Organizations, including the BioPhorum Operations Group (BPOG), Parenteral Drug Association (PDA), and

International Society for Pharmaceutical Engineering (ISPE), have spearheaded collaborative efforts to develop consensus-based best practices [8]. The PDA Technical Report 26 provides comprehensive guidance on filter validation and integrity testing, establishing science-based recommendations for test method selection, acceptance criteria determination, and deviation management [8]. Similarly, BPOG's Filter Integrity Testing Workstream has developed standardized protocols for both traditional biologics and advanced therapies, with documented adoption by numerous manufacturers resulting in harmonized practices across participating organizations. These initiatives have yielded tangible benefits, including reductions in filter failures attributed to procedural inconsistencies, decreased investigation times following test failures, and enhanced regulatory acceptance during inspections and submissions [8]. Emerging standards are addressing historical gaps in specific applications, such as virus filtration integrity verification and testing of filters integrated within single-use assemblies. Cross-industry data sharing initiatives facilitated by these organizations have established benchmark performance metrics for common filter types and testing methodologies, enabling more informed risk assessments and validation strategies. These harmonization efforts are particularly valuable for cell and gene therapy manufacturers navigating complex regulatory landscapes with limited precedent, providing science-based frameworks that satisfy diverse regulatory expectations while maintaining appropriate focus on patient safety and product quality [8]. Automated integrity testers and digital quality systems represent transformative technologies reshaping FIT implementation across biopharmaceutical manufacturing. Modern automated platforms—including Sartochek 5 Plus (Sartorius), Palltronic Flowstar (Pall), and Integritest 5 (Millipore)—offer sophisticated capabilities that enhance test sensitivity, reproducibility, and compliance [7]. These systems support multiple test methodologies (Bubble Point, Diffusion, Pressure Hold, Water Intrusion) with precise control of critical parameters such as pressure ramp rates, temperature compensation, and test duration. Advanced features include barcode scanning for filter identification, electronic signature capture compliant with 21 CFR Part 11, and real-time data trending to detect subtle performance shifts before failures occur [7]. Integration with Manufacturing Execution Systems (MES) and Laboratory Information Management Systems (LIMS) enables seamless data transfer, paperless documentation, and centralized record

management. This integration supports batch release decisions with comprehensive data visualization tools highlighting potential anomalies for investigation. Digital quality systems incorporating artificial intelligence (AI) algorithms can analyze historical test data to identify patterns predictive of future failures, enabling proactive intervention before critical batches are jeopardized [7]. Electronic workflow management ensures consistent execution of testing procedures, reducing operator variability through guided interfaces and automated calculations. Real-world implementations have demonstrated significant improvements in first-pass test success rates, reductions in investigation cycle times, and enhanced audit readiness through comprehensive electronic audit trails. These technological advances are particularly valuable in cell therapy manufacturing, where short shelf lives necessitate rapid, reliable testing with minimal procedural complexity [7]. Computational modeling and predictive approaches offer powerful tools for optimizing filter design, testing parameters, and failure mode analysis in biopharmaceutical applications. Computational Fluid Dynamics (CFD) simulations enable detailed visualization of fluid behavior within filter constructs, pressure distributions across membranes, and stress concentrations that may indicate potential failure points [8]. These models have been successfully validated against experimental data, demonstrating excellent correlation between predicted and observed bubble point pressures, diffusion rates, and pressure decay profiles [8]. By simulating various operating conditions—including worst-case scenarios involving maximum pressure differentials, temperature extremes, and challenging fluid properties—manufacturers can define appropriate test parameters that ensure filter integrity under actual use conditions rather than idealized laboratory environments. Digital twins of filtration systems, incorporating real-time sensor data from manufacturing operations, enable continuous monitoring of filter performance against predicted behavior, with deviations triggering alerts for potential integrity concerns [8]. Finite Element Analysis (FEA) models assess mechanical stresses on filter housings, support structures, and connection points during sterilization, installation, and operation, identifying vulnerable components that may compromise system integrity. These computational tools support risk-based validation strategies aligned with ICH Q9 principles by quantifying the relationship between filter characteristics, process parameters, and integrity test results [8]. Manufacturers leveraging these approaches have documented reductions in filter

failure rates during commercial operations, fewer deviations during validation exercises, and more robust justifications for test parameter selection in regulatory submissions. As models continue to increase in sophistication, their predictive power enhances both testing strategies and filter design optimization for challenging applications such as high-viscosity formulations and cell-containing products [8]. AI and machine learning applications are emerging as transformative technologies in the field of Filter Integrity Testing, offering unprecedented capabilities for anomaly detection, predictive maintenance, and continuous improvement. Advanced pattern recognition algorithms analyze integrity test data streams—including pressure curves, flow rates, and temperature profiles—to identify subtle deviations from expected behavior that may indicate incipient filter failures [7]. These systems leverage historical test results across multiple batches to establish baseline performance expectations for specific filter configurations, detecting anomalies that might escape traditional statistical process control methods [7]. Machine learning models trained on validated data sets can distinguish between genuine integrity concerns and procedural artifacts, reducing false alarms while maintaining high sensitivity to actual defects. Natural Language Processing (NLP) capabilities extract insights from unstructured data sources, including deviation reports, operator notes, and investigation findings, identifying recurring issues and facilitating knowledge transfer across manufacturing networks. Real-time monitoring systems incorporating AI analyze filter performance during actual use, correlating integrity test results with process parameters to identify conditions that stress filter systems and may compromise sterility assurance [7]. Digital assistants guide operators through complex testing procedures, adapting instructions based on filter type, test method, and facility-specific requirements to ensure consistent execution. Automated test optimization algorithms continuously refine test parameters—including pressure ramp rates, equilibration times, and endpoint determinations—to maximize sensitivity while minimizing false positives [7]. As these technologies mature, they are increasingly integrated into comprehensive Process Analytical Technology (PAT) frameworks supporting real-time release strategies for advanced therapies, where traditional quality control approaches may not accommodate limited shelf life and patient-specific manufacturing paradigms. While still evolving, early implementations have demonstrated promising results in reducing test failures attributable to procedural errors, enhancing

detection of genuine integrity breaches, and supporting more informed decision-making following ambiguous test results [7].

5. Standardization and Innovation

Regulatory interpretation disparities present significant challenges in Filter Integrity Testing (FIT) implementation across biopharmaceutical manufacturing networks. While FDA's 21 CFR Part 211, EU GMP Annex 1, and USP <797> all mandate filter integrity verification, their specific requirements vary considerably, creating ambiguity for global manufacturers [9]. The EU GMP Annex 1 explicitly requires Pre-Use Post-Sterilization Integrity Testing (PUPSIT) for all sterilizing filters, while the FDA has adopted a more risk-based approach, allowing alternative strategies based on product criticality. These divergent interpretations create operational complexities for multinational organizations, with manufacturers maintaining distinct protocols for products destined for different markets. The implications extend beyond procedural differences to impact equipment selection, documentation requirements, and validation strategies. Regulatory guidance documents from the FDA and EMA offer differing perspectives on testing methodologies, acceptance criteria, and failure investigations [9]. Contract development and manufacturing organizations face particular challenges in navigating diverse client expectations while maintaining consistent quality systems, potentially creating compliance vulnerabilities during regulatory inspections. Industry harmonization initiatives establish standardized FIT approaches that satisfy global regulatory expectations while enabling operational efficiency. Organizations, including BioPhorum Operations Group, Parenteral Drug Association, and International Society for Pharmaceutical Engineering, have developed consensus-based best practices [10]. PDA Technical Report 26 provides comprehensive guidance on filter validation and integrity testing, with science-based recommendations for test method selection, acceptance criteria, and deviation

management. BPOG's Filter Integrity Testing Workstream has developed standardized protocols for both traditional biologics and advanced therapies, with documented adoption across numerous manufacturers [10]. These initiatives have reduced filter failures attributed to procedural inconsistencies, decreased investigation times, and enhanced regulatory acceptance during inspections. Emerging standards address historical gaps in specific applications, such as virus filtration integrity verification and testing filters integrated within single-use assemblies. Cross-industry data sharing has established benchmark performance metrics for common filter types, enabling more informed risk assessments and validation strategies, particularly valuable for cell therapy manufacturers navigating complex regulatory landscapes. Automated integrity testers and digital quality systems transform FIT implementation across biopharmaceutical manufacturing. Modern platforms—including Sartochek 5 Plus, Palltronic Flowstar, and Integritest 5—enhance test sensitivity, reproducibility, and compliance [9]. These systems support multiple test methodologies with precise control of critical parameters, including pressure ramp rates, temperature compensation, and test duration. Advanced features include barcode scanning, electronic signatures compliant with 21 CFR Part 11, and real-time data trending. Integration with Manufacturing Execution Systems and Laboratory Information Management Systems enables seamless data transfer and centralized record management, supporting batch release decisions with comprehensive visualization tools highlighting potential anomalies. Digital quality systems incorporating artificial intelligence analyze historical test data to identify patterns predictive of future failures, enabling proactive intervention [9]. Electronic workflow management ensures consistent testing procedures through guided interfaces and automated calculations, with implementations demonstrating improvements in test success rates, reduced investigation cycles, and enhanced audit readiness—particularly valuable in cell therapy manufacturing where short shelf lives necessitate rapid, reliable testing.

Table 1: Filter Integrity Testing Methods and Applications in Biopharmaceutical Manufacturing [3, 4]

Test Method	Application	Technical Considerations
Bubble Point Test	Primarily used for hydrophobic filters; measures the pressure at which continuous bubbling occurs through a wetted membrane; can detect defects as small as 2-3 micrometers	Requires the highest pressure among common methods; provides definitive visual confirmation of integrity breaches; most susceptible to wetting issues
Diffusion Test	Ideal for hydrophilic filters; measures gas diffusion rate through wetted membrane under pressure below bubble point; can	More sensitive than bubble point for detecting minor defects; temperature fluctuations can significantly alter

	identify pinholes less than 1 micrometer in diameter	results; requires precise calibration
Pressure Hold Test	Variation of diffusion testing; monitors pressure drop over time on the upstream side of the wetted filter; particularly valuable for filters with low flow rates	Useful when automated systems are unavailable; less sensitive than automated diffusion testing; environmental conditions affect reliability
Water Intrusion Test (WIT)	Specifically designed for hydrophobic filters used in gas filtration or venting applications; measures water penetration under pressure	Verifies membrane integrity without wetting the filter; requires specialized equipment; less common but highly effective for PTFE filters
Automated Integrity Testers	Support multiple test methodologies with precise control of critical parameters; examples include Sartocheck 5 Plus, Palltronic Flowstar, and Millipore Integritest 5	Offer electronic data capture; integration with MES/LIMS systems; reduce operator variability; enable real-time trending and regulatory compliance

Table 2: Industry Forum Recommendations Addressing Cell Therapy FIT Challenges [5, 6]

Table 2 Challenge	Specific Forum Recommendation	Expected Benefit
Closed-System Constraints	BPOG standardized protocols for single-use assemblies	Alternative testing strategies validated for closed systems
Technical Limitations	PDA TR26 science-based test method selection criteria	Reduced method variability through standardized approaches
Filter-Specific Validation	Emerging standards for virus filtration and specialized filters	Validated methods for diverse filter configurations

Table 3: Cell Therapy Implementation Challenges for Filter Integrity Testing [5, 6]

Challenge Category	Description	Impact on Manufacturing
Closed-System Constraints	Single-use, closed-system designs significantly restrict access to filters post-use; manufacturers face major obstacles in performing Post-Use FIT, creating greater reliance on Pre-Use and PUPSIT testing	Limited testing options; increased risk of undetected filter failures; more complex validation strategies required to ensure sterility assurance
Technical Limitations	Traditional methods like bubble point testing cannot detect micro-defects smaller than a few micrometers; method variability leads to inconsistent results across facilities; manual interpretation introduces subjectivity	Reduced confidence in test results; requirement for redundant testing strategies; increased resource consumption; potentially compromised product sterility
Operator Training Deficiencies	Human factors, including inconsistent test setups and procedural deviations, contribute significantly to result variability; technicians often lack extensive experience with filtration systems compared to traditional biopharmaceutical operators	High proportion of FIT-related deviations attributable to operator technique rather than actual filter failures; knowledge gaps in proper filter handling
Equipment Qualification Gaps	Many facilities conduct Installation Qualification (IQ) and Operational Qualification (OQ), but lack robust Performance Qualification (PQ) protocols specific to cell therapy applications	Inadequate verification of system performance under actual operating conditions; increased risk of unexpected failures during manufacturing
Filter-Specific Validation	Cell therapies utilize diverse filter types and configurations requiring customized testing approaches; many facilities use generic methods that fail to detect breaches in specific filter configurations	Multi-layer filters may pass bubble point testing despite harboring defects in internal layers; cryopreserved product filtration introduces unique challenges requiring specialized validation

Table 4: Standardization and Innovation Approaches in Filter Integrity Testing [7, 8]

Innovation Category	Key Features	Implementation Benefits
Regulatory Harmonization	Organizations like BioPhorum, PDA, and ISPE are developing consensus-based best	Reduction of operational complexities for global manufacturers; enhanced

Initiatives	practices; PDA Technical Report 26 guides filter validation and integrity testing	regulatory acceptance during inspections; science-based frameworks satisfying diverse regulatory expectations
Automated Integrity Testers	Modern platforms (Sartocheck 5 Plus, Palltronic Flowstar, Integritytest 5) with precise control of critical parameters, barcode scanning, electronic signature capture, and real-time data trending	Enhanced test sensitivity and reproducibility; reduced operator variability; improved documentation for regulatory compliance; faster testing for short shelf-life products
Digital Quality Systems	Integration with MES/LIMS; paperless documentation; centralized record management; comprehensive data visualization tools; electronic workflow management	Seamless data transfer; enhanced batch release decisions; consistent execution of testing procedures; improved audit readiness; reduced investigation cycle times
Computational Modeling	CFD simulations visualizing fluid behavior and pressure distributions; digital twins incorporating real-time sensor data; Finite Element Analysis for mechanical stress assessment	Appropriate test parameter definition for actual use conditions; continuous monitoring capabilities; identification of vulnerable components; support for risk-based validation strategies
AI and Machine Learning	Pattern recognition algorithms analyzing test data streams; models distinguishing genuine concerns from procedural artifacts; Natural Language Processing for unstructured data analysis; automated test optimization	Early detection of potential failures; reduced false alarms while maintaining sensitivity; knowledge transfer across manufacturing networks; support for real-time release strategies

Table 5: Regulatory and Technological Innovations in Filter Integrity Testing [9, 10]

Category	Key Elements	Impact on Biopharmaceutical Manufacturing
Regulatory Disparities	Differences between FDA (risk-based approach) and EU GMP Annex 1 (mandatory PUPSIT); varying requirements for documentation and validation	Creates operational complexities for global manufacturers; necessitates market-specific protocols; increases compliance challenges during inspections
Industry Harmonization Efforts	PDA Technical Report 26; BioPhorum Operations Group workstreams; consensus-based best practices	Standardized protocols reduce procedural inconsistencies, enhance regulatory acceptance, and improve guidance for cell therapy manufacturers
Automated Testing Platforms	Sartocheck 5 Plus; Palltronic Flowstar; Integritytest 5; precise control of critical parameters	Enhanced test sensitivity and reproducibility; reduced operator variability; improved documentation for compliance; faster testing for short-shelf-life products
Digital Quality Systems	Electronic signature capture; real-time data trending; integration with MES/LIMS; centralized record management	Seamless data transfer; improved batch release decision support; reduced investigation cycle times; enhanced audit readiness
AI and Predictive Technologies	Pattern recognition algorithms; historical data analysis; automated parameter optimization	Proactive identification of potential failures; distinction between genuine concerns and procedural artifacts; support for real-time release strategies

4. Conclusions

Filter Integrity Testing is also a key control level in biopharmaceutical manufacturing that has a direct effect on product sterility, patient safety, and regulatory compliance. Testing modalities: With the industry moving towards complex modalities such as cell and gene therapies, there are

restrictions to the existing ways of testing that demand novel solutions. Implementing robotized testers, computerized quality control, and computer-based modeling allows the verification to be more stable, as well as decreasing operator variability. Efforts to harmonize the industry have been effective in standardizing practices in the global networks of manufacturers, but differences

in regulatory interpretation are still a challenge. In the case of cell therapy specifically, closed-system limitations and time-sensitive issues require specific solutions with unique validation concerns. With the development of AI and machine learning applications, it is expected that there will be improved detection of anomalies and predictive abilities that can be used in real-time monitoring and preventive measures. Using extensive testing approaches that consider filter-specific properties, environmental conditions, and operational limits, manufacturers would be able to provide sterility assurance and, at the same time, allow the manufacturability of higher-order therapeutic products.

Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
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