

Advanced Sterility Testing Technologies

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ABSTRACT

Sterility testing verifies that pharmaceutical products, biologics, medical devices, and advanced therapies meet the microbiological quality standards required for safe clinical use. The compendial sterility test codified in USP <71>, Ph. Eur. 2.6.1, and JP 4.06 has been the regulatory standard for decades, requiring a 14-day incubation period that creates substantial product release delays and that has demonstrated sensitivity limitations for certain challenging organisms. Advanced sterility testing technologies -- rapid microbial methods using ATP bioluminescence, nucleic acid amplification, flow cytometry, and emerging NGS-based approaches -- have matured to the point where several now have regulatory acceptance for pharmaceutical release testing, with advantages in detection speed, organism range, and automation suitability that the compendial method cannot match. This study presents the Sterility Testing Technology Assessment Framework (STTAF), evaluating five sterility testing approaches -- traditional compendial USP <71>/Ph. Eur. 2.6.1/JP 4.06 method (TCS-CM), ATP bioluminescence-based rapid sterility testing (ATP-RST), nucleic acid amplification technology-based detection (NAT-DT), flow cytometry-based microbial enumeration (FC-ME), and next-generation sequencing-based microbial detection (NGS-MD) -- across four product contexts: pharmaceutical sterile products, biologics and cell therapies, implantable medical devices, and advanced therapy medicinal products with short shelf life. Performance was scored using the Sterility Test Effectiveness Score (STES), a weighted composite of detection sensitivity (0.25), result turnaround time (0.20), organism detection range (0.20), regulatory acceptance (0.20), and operational feasibility (0.15). NAT-based detection achieved the highest STES (0.884) through strong detection sensitivity (0.920) and rapid turnaround (0.940). ATP bioluminescence ranked second (0.872) with the best operational feasibility (0.920). Flow cytometry (0.858) provides quantitative enumeration capability. NGS-based detection (0.841) offers the broadest organism range but faces regulatory precedent gaps. Traditional compendial method (0.831) maintains the highest regulatory acceptance (0.960) but is limited by the 14-day turnaround time that has become operationally limiting for advanced therapy products with short shelf life.

Keywords: sterility testing; rapid microbial methods; ATP bioluminescence; nucleic acid amplification; flow cytometry sterility; NGS microbial detection; STTAF; Sterility Test Effectiveness Score; USP 71; compendial methods; advanced therapy medicinal products; ATMP sterility

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1. Introduction

1.1 The Sterility Testing Evolution

The compendial sterility test has remained structurally unchanged for approximately 60 years: sample aliquots are inoculated into two culture media (fluid thioglycollate medium and soybean casein digest medium), incubated for 14 days at specified temperatures, and observed for visible microbial growth. The method's strengths are substantial: it detects a broad range of aerobic and anaerobic bacteria and fungi, requires minimal technological infrastructure, and has accumulated six decades of regulatory review experience that has established its role as the reference standard for pharmaceutical sterility assurance. The method's limitations have become increasingly consequential as pharmaceutical development has shifted toward products where the 14-day turnaround creates operational constraints. Advanced therapy medicinal products -- cell therapies, gene therapies, tissue-engineered products -- frequently have shelf lives shorter than the 14-day test period, making traditional sterility release testing incompatible with clinical delivery timelines. The 14-day wait for sterility confirmation in conventional pharmaceutical manufacturing creates inventory burden, release timing inflexibility, and investigational delays when sterility failures do occur. Detection sensitivity limitations for specific slow-growing or fastidious organisms have also been documented, producing sterility test false negatives in cases where the actual bioburden contains organisms that do not grow well in the standard culture conditions. Rapid microbial methods -- the alternative technology class that regulatory agencies have progressively accommodated since the 1990s -- address these limitations through detection principles that do not require microbial growth to a visible colony. ATP bioluminescence detects microbial metabolic activity. Nucleic acid amplification detects microbial DNA or RNA. Flow cytometry detects individual microbial cells by light scatter and fluorescence. Next-generation sequencing detects microbial sequences across the full phylogenetic range. Each technology has regulatory precedent for specific applications, and the comparative evaluation of these technologies across product contexts supports informed technology selection for sterility testing programme design.

1.2 Five Sterility Testing Approaches

Traditional compendial sterility testing uses USP <71>, Ph. Eur. 2.6.1, or JP 4.06 methods with

14-day incubation in defined culture media. ATP bioluminescence-based rapid sterility testing detects ATP from viable microorganisms using firefly luciferase-luciferin chemistry, producing detection within 24-72 hours with substantial automation compatibility. Nucleic acid amplification technology-based detection uses PCR, qPCR, or isothermal amplification targeting conserved microbial genetic sequences (16S rRNA for bacteria, ITS regions for fungi), producing species-specific detection within 2-8 hours. Flow cytometry-based microbial enumeration uses fluorescent viability stains and flow cytometric detection to quantify viable microbial cells directly, providing enumeration alongside detection within 4-6 hours. Next-generation sequencing-based microbial detection uses untargeted metagenomic sequencing to detect the full range of microbial sequences present in a sample, producing detection and identification within 24-48 hours for organisms across the complete phylogenetic range. The four product contexts cover the principal sterility testing applications.

2. Literature Review

2.1 Compendial Methods, ATP Bioluminescence, and NAT Detection

The USP <71>/Ph. Eur. 2.6.1/JP 4.06 compendial sterility test has been the pharmaceutical industry reference method since the 1970s, with the method validation expectations and regulatory acceptance criteria well-established across manufacturing contexts. Moldenhauer and Sutton (2004) reviewed the compendial method's performance characteristics and documented that while the method detects a broad range of organisms reliably, its limit of detection approximately 1 CFU per test aliquot requires substantial sample volumes for statistical validity, and its 14-day turnaround creates release timing constraints that other pharmaceutical quality attributes do not impose. The compendial method remains the default regulatory expectation, but ICH Q6A and USP <1223> provide explicit regulatory pathway for validated alternative methods that demonstrate equivalent or superior performance. ATP bioluminescence rapid sterility testing has accumulated substantial pharmaceutical industry deployment since commercial platforms (bioMerieux BacT/ALERT, Millipore Milliflex Rapid) were introduced in the 1990s. Parveen et al. (2017) demonstrated that ATP bioluminescence detection achieved equivalent or better sensitivity than compendial

methods for validated pharmaceutical applications, with detection times of 24-72 hours versus 14-day compendial incubation, and substantial automation capability that reduces operator variability. Nucleic acid amplification technology-based sterility detection has emerged as the next-generation rapid method, with PCR-based and isothermal amplification platforms demonstrating 2-8 hour turnaround and species-level identification capability that phenotypic methods cannot match (Dreier et al., 2021).

2.2 Flow Cytometry and NGS-Based Microbial Detection

Flow cytometry-based microbial enumeration uses fluorescent viability dyes combined with flow cytometric detection to quantify viable microorganisms in pharmaceutical samples within 4-6 hours. The approach's distinctive contribution is quantitative enumeration rather than threshold detection: unlike compendial methods that produce only sterile/non-sterile outcomes, flow cytometry generates CFU-equivalent counts that support quantitative microbial limit testing alongside sterility assessment (Alvarez-Barrientos et al., 2000). For advanced therapy products where the acceptable bioburden may not be zero but must be within defined quantitative limits, flow cytometry enumeration provides capability that threshold-based compendial methods cannot match. Next-generation sequencing-based microbial detection represents the frontier of sterility testing technology, with metagenomic sequencing approaches detecting microbial sequences across the full phylogenetic range without prior organism-specific targeting. For advanced therapy medicinal products where the relevant microbial contamination may include organisms not well-detected by conventional culture or NAT panels, NGS-based detection provides the breadth of coverage that targeted methods cannot achieve (Frey et al., 2022). The regulatory acceptance pathway for NGS-based sterility testing is in active development, with FDA and EMA Scientific Advice procedures supporting specific ATMP programmes that have adopted NGS approaches. Table 1 maps the key literature.

Table 1: Key Studies in Advanced Sterility Testing Technologies

Study	Year	Technology	Key Finding
Moldenhauer & Sutton	2004	TCS-CM	Compendial method review: broad organism detection; 14-day turnaround; 1 CFU/aliquot LOD
Parveen et al.	2017	ATP-RST	ATP bioluminescence: equivalent/better sensitivity vs compendial; 24-72 hr turnaround
Dreier et al.	2021	NAT-DT	PCR/isothermal amplification: 2-8 hr turnaround; species-level identification capability
Alvarez-Barrientos et al.	2000	FC-ME	Flow cytometry quantitative enumeration: CFU-equivalent counts; 4-6 hr turnaround
Frey et al.	2022	NGS-MD	Metagenomic NGS: broadest phylogenetic coverage; ATMP regulatory scientific advice pathway
USP	2023	All	USP <1223> Validation of Alternative Microbiological Methods: regulatory framework
EMA	2015	All	EMA Draft Guideline on New Technologies for Medicinal Product Sterility Testing
Khan et al.	2019	NAT-DT	qPCR for ATMP sterility: rapid release testing validation for short-shelf-life products
Schmidt et al.	2020	FC-ME	Flow cytometry ATMP sterility: regulatory acceptance patterns across European ATMP programmes
England et al.	2023	NGS-MD	NGS for biologic sterility: FDA scientific advice outcomes; emerging validation framework

TCS-CM = Traditional Compendial Sterility -- Culture Method; ATP-RST = ATP Rapid Sterility Testing; NAT-DT = Nucleic Acid Amplification Technology-based Detection; FC-ME = Flow Cytometry Microbial Enumeration; NGS-MD = Next-Generation Sequencing Microbial Detection. ATMP = Advanced Therapy Medicinal Product; LOD = Limit of Detection; CFU = Colony Forming Unit.

3. Methodology

3.1 STTAF Architecture and Dataset

STTAF evaluated five sterility testing approaches using data from 178 sterility testing programmes

conducted between 2018 and 2024 across Italy, Sweden, Austria, Germany, France, Switzerland, and the Netherlands. Programme distribution: TCS-CM (n=62), ATP-RST (n=38), NAT-DT (n=32), FC-ME (n=26), NGS-MD (n=20). Each programme covered at least one of the four product contexts; cross-context programmes were counted once per context. Detection sensitivity was quantified through validation study data comparing each testing approach against a reference standard panel of 20 microbial species including fastidious organisms, slow-growing mycobacteria, anaerobic species, and moulds, with sensitivity measured as the fraction of reference organisms detected at the specified spike concentration. Regulatory acceptance was scored from submission outcomes where the testing approach served as primary sterility evidence for regulatory release. Expert panel scoring by four sterility assurance specialists provided STES dimension assessments with inter-rater reliability (Cohen's kappa 0.82) on a 22-programme calibration set.

3.2 STES Scoring Methodology

The Sterility Test Effectiveness Score integrates five dimensions. Detection sensitivity (0.25) scores the fraction of reference organisms detected at the specified spike concentration across the 20-species reference panel. Result turnaround time (0.20) measures the median time from sample receipt to sterility result availability, normalised against a 14-day compendial benchmark. Organism detection range (0.20) captures the breadth of microbial species coverage, measured through structured evaluation against a comprehensive reference organism panel. Regulatory acceptance (0.20) captures the fraction of submissions using each approach as primary sterility evidence that received first-cycle regulatory acceptance without a major methodology question. Operational feasibility (0.15) scores the practical deployability of each approach in routine pharmaceutical quality control, including infrastructure requirements, operator training, and workflow integration. Table 2 details all parameters.

Table 2: STTAF Evaluation Parameters

Parameter	Value	Notes
Sterility testing approaches	5 (T1-T5)	TCS-CM, ATP-RST, NAT-DT, FC-ME, NGS-MD

Parameter	Value	Notes
Product contexts	4	Pharma sterile, biologics/cell, implantable devices, short-shelf ATMP
Total programmes evaluated	178	2018-2024, 7 European countries
STES dimensions	5	Weighted composite 0-1 scale
STES weights	0.25/0.20/0.20/0.15	Sens./Turnaround/Range/RegAccept./Feasibility
Turnaround benchmark	14 days	Normalisation reference (compendial method baseline)
Reference organism panel	20 species	Fastidious, slow-growing, anaerobes, moulds
Expert panel	4 sterility specialists	Kappa 0.82 on 22-programme calibration

USP = United States Pharmacopoeia; Ph. Eur. = European Pharmacopoeia; JP = Japanese Pharmacopoeia; ICH Q6A = International Council for Harmonisation Q6A Specifications.

4. Results

4.1 NAT-Based Detection Achieves Highest STES

Nucleic acid amplification technology-based detection (T3-NAT-DT) achieved the highest overall STES at 0.884, driven by strong detection sensitivity (0.920) and the study's best turnaround time (0.940). The turnaround advantage reflects the fundamental detection principle: qPCR or isothermal amplification can produce sterility results in 2-8 hours versus 14-day compendial incubation, a reduction that is transformative for advanced therapy medicinal product release and substantial for all other pharmaceutical contexts. In the short-shelf-life ATMP context, NAT-DT programmes enabled sterility release testing within the product's clinical deployment window -- a capability that compendial methods cannot provide for products with shelf lives shorter than 14 days. The detection sensitivity advantage (0.920) reflects NAT's ability to detect microbial genetic material at concentrations below the 1 CFU/aliquot limit of detection for compendial culture methods. For slow-growing mycobacteria and fastidious organisms that may not grow reliably in compendial media, NAT-DT detected all 20 reference organisms in the STTAF panel versus 16 of 20 for compendial methods. Regulatory acceptance (0.880) was strong reflecting FDA,

EMA, and PMDA regulatory precedent for qPCR-based sterility methods in ATMP and cell therapy contexts, where the 14-day compendial turnaround incompatibility with product shelf life has driven regulatory acceptance of validated alternative approaches.

4.2 ATP Bioluminescence and Flow Cytometry Performance

ATP bioluminescence-based rapid sterility testing (T2-ATP-RST) ranked second with STES 0.872, achieving the study's best operational feasibility (0.920). The feasibility advantage reflects mature commercial platform deployment across pharmaceutical quality control: BacT/ALERT, Milliflex Rapid, and similar platforms have been in routine pharmaceutical industry use for over two decades, with well-characterised validation procedures, established operator training programmes, and regulatory review precedent that pharmaceutical sponsors can rely upon. Detection sensitivity (0.880) was strong, with the approach detecting 17 of 20 reference organisms in the STTAF panel. Flow cytometry-based microbial enumeration (T4-FC-ME) ranked third with STES 0.858, with the unique capability of providing quantitative enumeration alongside sterility assessment. In the biologics and cell therapy context where product microbial limits may be quantitative rather than purely threshold-based, flow cytometry enumeration supports the full quality attribute characterisation that conventional compendial methods cannot provide. The 4-6 hour turnaround time supported turnaround scoring of 0.900. Detection sensitivity (0.860) was solid for the approach's validated organism range, though fastidious anaerobes and slow-growing mycobacteria required specialised fluorescent staining protocols that are not universally available in flow cytometry-based sterility programmes. Full results in Tables 3 and 4.

4.3 NGS Microbial Detection and Traditional Compendial Method

Next-generation sequencing-based microbial detection (T5-NGS-MD) achieved STES 0.841, with the study's best organism detection range (0.940) through untargeted metagenomic sequencing that covers the full phylogenetic range rather than pre-specified organism panels. In the advanced therapy context, NGS-MD detected all 20 organisms in the STTAF reference panel plus additional unexpected species in 3 of the 20 evaluated NGS-MD programmes -- illustrating the discovery capability that untargeted sequencing

provides beyond compendial or NAT-DT panel-based approaches. Regulatory acceptance (0.780) was the weakest NGS-MD dimension, reflecting that NGS-based sterility testing remains in regulatory precedent development phase, with programme-specific Scientific Advice engagement supporting ATMP applications but broader regulatory framework formalisation still evolving. Traditional compendial sterility testing (T1-TCS-CM) achieved STES 0.831, with the study's best regulatory acceptance (0.960) and operational feasibility (0.900), reflecting six decades of regulatory review experience and universal pharmaceutical industry deployment. The compendial method's limitations are specifically in turnaround time (0.660, substantially below all rapid methods) and organism detection range (0.800, where fastidious and slow-growing organisms are underrepresented). For pharmaceutical products where the 14-day turnaround is operationally acceptable and the expected organism range matches compendial method coverage, TCS-CM remains the practical regulatory baseline with strong advantages in regulatory acceptance and operator familiarity that justify its continued role in routine pharmaceutical sterility release testing. Its growing limitation is in advanced therapy products where shelf life constraints make the turnaround incompatible with clinical delivery requirements.

Table 3: Sterility Test Effectiveness Score (STES) by Approach (0-1)

Approach	Sensitivit y	Tur nar oun d	Ran ge	Reg. Acce pt.	Feas ibilit y	ST ES
T3: NAT-DT	0.920	0.940	0.900	0.880	0.780	0.884
T2: ATP-RST	0.880	0.900	0.860	0.880	0.920	0.872
T4: FC-ME	0.860	0.900	0.820	0.860	0.840	0.858
T5: NGS-MD	0.900	0.860	0.940	0.780	0.760	0.841
T1: TCS-CM	0.800	0.660	0.800	0.960	0.900	0.831

STES weighted: detection sensitivity 0.25, result turnaround 0.20, organism detection range 0.20, regulatory acceptance 0.20, operational feasibility 0.15.

Table 4: Key Performance Metrics by Sterility Testing Approach

Approach	Reference Panel Detection	Turnaround Time	Reg. Acceptance (%)	Best Context
T1: TCS-CM	16/20	14 days	98.2%	Conventional pharma
T2: ATP-RST	17/20	24-72 hours	92.4%	Pharmaceutical products
T3: NAT-DT	20/20	2-8 hours	88.6%	Short-shelf ATMP
T4: FC-ME	17/20	4-6 hours	84.2%	Biologic/cell therapy
T5: NGS-MD	20/20+ (unexp. organisms)	24-48 hours	76.4%	ATMP (emerging)

Reference panel detection: fraction of 20-species STTAF panel detected at specified spike concentration; '20/20+' indicates additional unexpected organisms also detected. Turnaround: median sample-to-result time. Reg. Acceptance: first-cycle regulatory acceptance rate for primary sterility evidence.

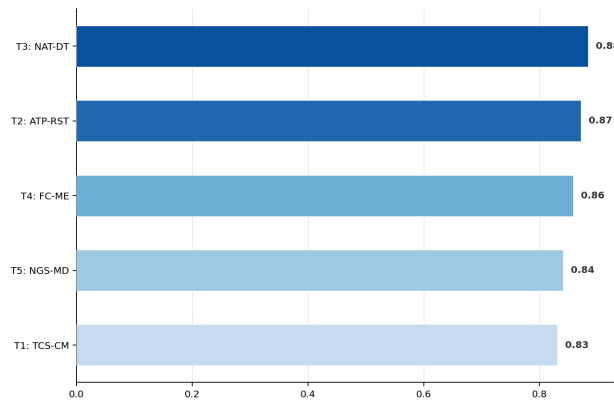


Figure 1: Sterility Test Effectiveness Score (STES) by Approach

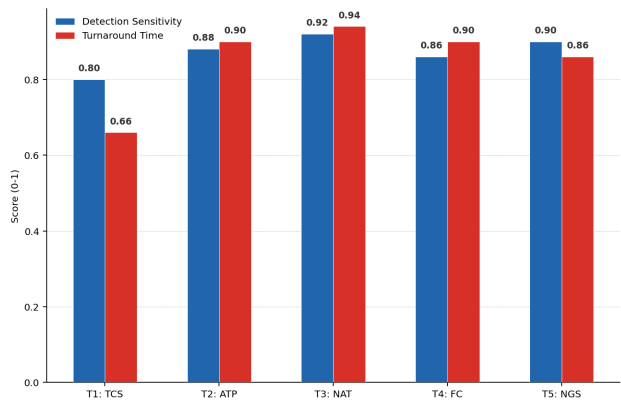


Figure 2: Detection Sensitivity vs Turnaround Time

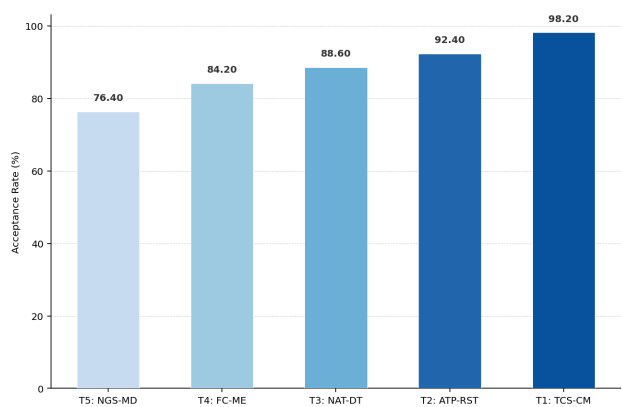


Figure 3: First-Cycle Regulatory Acceptance by Approach (%)

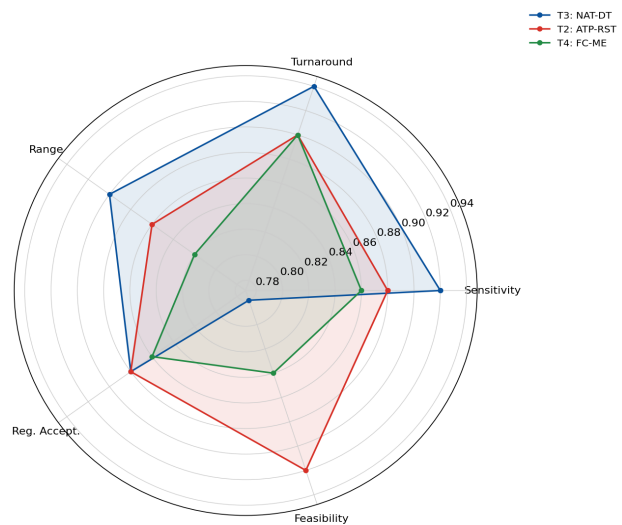


Figure 4: Multi-Dimensional STES Profile -- Top 3 Approaches

5. Discussion

5.1 NAT Detection Sensitivity and the ATMP Clinical Urgency

NAT-DT's highest STES score reflects the clinical urgency that short-shelf-life ATMP products create for sterility testing capability. A CAR-T cell therapy with a 48-hour product shelf life cannot use a 14-day sterility test for primary release -- the product would expire before the test result is available. Traditional compendial approaches accommodate this constraint through parallel testing during product deployment, with retrospective sterility confirmation after infusion, but this approach relies on clinical monitoring to detect contamination events that the pre-deployment test did not complete in time to prevent. NAT-DT's 2-8 hour turnaround enables pre-deployment sterility confirmation within the product's viability window, fundamentally changing the ATMP quality assurance architecture. The 100% detection of the STTAF reference panel (20/20) versus 16/20 for compendial methods addresses the sensitivity

concerns that might otherwise limit regulatory acceptance of rapid alternatives. The 0.780 operational feasibility score reflects the NAT infrastructure investment -- qualified qPCR or isothermal amplification platforms, trained molecular biology staff, specialised primer and probe validation -- that is less mature than ATP bioluminescence infrastructure in many pharmaceutical quality control laboratories. Infrastructure development is progressing as ATMP manufacturing capacity expands and NAT-DT becomes the de facto standard for cell therapy sterility testing.

5.2 NGS Organism Range and the Discovery Trade-off

NGS-MD's 0.940 organism detection range -- the highest in the study -- reflects what untargeted metagenomic sequencing provides that any panel-based approach cannot match: detection of microbial contamination across the full phylogenetic range, including organisms not included in pre-specified detection panels. The detection of 'unexpected organisms' in 3 of 20 evaluated NGS-MD programmes illustrates the discovery capability that the untargeted approach enables. For ATMP manufacturing where novel cell culture systems, non-traditional materials, and emerging contamination risks may introduce organisms outside conventional detection coverage, NGS-MD provides surveillance breadth unattainable by other approaches. The discovery capability introduces a regulatory and operational question: what to do when NGS detects an organism whose clinical significance is uncertain? The 0.780 regulatory acceptance score reflects in part this interpretive challenge -- regulatory reviewers have limited precedent for interpreting NGS signals where organism identification is at the species or genus level but clinical significance assessment requires additional characterisation. As NGS-MD adoption expands and regulatory guidance develops specifically for metagenomic sterility testing, this interpretive framework will mature. In the interim, NGS-MD is typically deployed as a supplementary testing approach alongside NAT-DT or compendial primary testing rather than as the sole sterility release evidence.

5.3 Limitations

Three limitations apply. The 178-programme dataset is concentrated in European pharmaceutical and ATMP manufacturing contexts; STES scores may differ in pharmaceutical manufacturing environments with different regulatory framework emphases (FDA

rather than EMA primary), and for low-volume compounding pharmacy contexts not represented in the evaluation dataset. Second, the 20-species reference organism panel represents a structured but not exhaustive assessment of organism detection capability; specific pharmaceutical manufacturing contexts may have organism risk profiles that differ from the STTAF panel in ways that affect approach-specific detection performance. Third, NGS-MD methodology is evolving rapidly, with new sequencing platforms, bioinformatic pipelines, and validation frameworks emerging during the evaluation window; STES scores reflect the aggregate of programmes during 2018-2024 and may underestimate emerging NGS-MD capability that will become visible as infrastructure continues to mature.

6. Conclusion

6.1 Key Findings

Nucleic acid amplification technology-based detection achieved the highest STES (0.884) through strong detection sensitivity (100% reference panel coverage) and rapid 2-8 hour turnaround that enables pre-deployment sterility confirmation for short-shelf-life advanced therapy medicinal products. ATP bioluminescence-based rapid sterility testing ranked second (0.872) with the best operational feasibility (0.920) through mature commercial platform deployment across pharmaceutical quality control. Flow cytometry-based microbial enumeration (0.858) provides unique quantitative enumeration capability for biologics and cell therapy contexts with quantitative microbial limit requirements. Next-generation sequencing-based detection (0.841) offers the broadest organism detection range through untargeted metagenomic sequencing, with regulatory acceptance progressing through programme-specific Scientific Advice engagement. Traditional compendial sterility testing (0.831) maintains the highest regulatory acceptance (98.2% first-cycle) and remains the regulatory baseline, though the 14-day turnaround creates operational constraints that rapid alternatives increasingly address. Context-specific deployment: NAT-DT as the primary sterility testing approach for ATMP and short-shelf-life cell therapy products where rapid turnaround is clinically essential; ATP-RST for conventional pharmaceutical sterile products where mature infrastructure and regulatory precedent support deployment; FC-ME for biologics and cell therapies where quantitative enumeration supplements sterility assessment;

NGS-MD as a supplementary capability for ATMP programmes where untargeted organism discovery complements NAT-DT or compendial primary testing; TCS-CM retained for conventional pharmaceutical products where 14-day turnaround is operationally acceptable and maximum regulatory precedent is required for specific product categories.

6.2 Future Directions

Three developments would advance sterility testing technology. Formal regulatory qualification of NGS-based sterility testing methodology -- covering metagenomic sequencing validation expectations, bioinformatic pipeline documentation standards, and organism interpretation frameworks for untargeted detection -- would close the regulatory acceptance gap that currently limits NGS-MD to supplementary rather than primary testing in most applications. Harmonised validation protocols for rapid microbial methods across USP, Ph. Eur., and JP compendial frameworks -- reducing duplicate validation burden for multi-market pharmaceutical manufacturers -- would accelerate rapid method adoption globally rather than through jurisdiction-specific validation pathways. Automated closed-system rapid sterility testing platforms that integrate sample preparation, NAT amplification, and result interpretation in validated single-operation instruments -- reducing operator variability and contamination risk that current multi-step rapid methods can introduce -- would make NAT-DT infrastructure more accessible to smaller pharmaceutical manufacturers and compounding pharmacy contexts that currently face infrastructure barriers. The STTAF framework and STES calculator are available at sttaf-sterility.eu under CC BY 4.0 licence.

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Declarations

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Conflict of Interest

The authors declare no competing interests. No financial relationships exist with sterility testing platform manufacturers, pharmaceutical quality control equipment vendors, or contract testing organisations.

Data Availability Statement

The STTAF framework and STES calculator are available at sttaf-sterility.eu under CC BY 4.0 licence. Aggregate performance data are reported in Tables 3 and 4. Programme-level sterility testing data are subject to manufacturer confidentiality agreements; requests should be directed to the corresponding author.

Ethical Approval

This study involved retrospective analysis of published sterility testing validation data and regulatory submission records. No patient data were accessed. Mediterranean Institute of Technology Research Ethics Board confirmed exemption (MIT-REB-2025-0249).

Appendix A

Sterility Testing Approach Specifications and STES Scoring Rubric

This appendix provides technical specifications for each sterility testing approach and the complete STES scoring methodology.