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Microbiological risks and biofilms on instruments/surfaces: validation of disinfection/sterilization protocols

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Abstract

The study presents a comprehensive examination of microbiological threats associated with the formation of microbial biofilms on the working surfaces of medical instruments and equipment in the healthcare realities of 2024–2025. On the basis of updated information from international organizations, as well as a synthesis of specialized publications indexed in Scopus and Web of Science, the analysis elucidates the problem of heightened resistance of biofilm communities to standard disinfection and sterilization regimens. Key strategies by which microorganisms maintain viability within the extracellular polymeric matrix are described in detail, including mechanical protection, restricted diffusion of antimicrobial factors, and the development of heterogeneous microzones with altered metabolic activity. In parallel, the dynamics and statistical regularities of healthcare-associated infections (HAIs) at the global level are examined, enabling biofilm-associated persistence mechanisms to be linked to epidemiological trends. A separate emphasis is placed on the transformation of international regulation and practice-oriented requirements for the reprocessing of medical devices, including the release of updated standards in the ISO 15883:2024/2025 series and their significance for evidence-based validation of technological processes in central sterilization departments. Within a comparative analysis, contemporary approaches to detecting biological contamination are considered, including ATP bioluminescence and fluorescent visualization methods, which are characterized by differing sensitivity and applied diagnostic value at stages of cleanliness control. The effectiveness of innovative enzymatic detergents in acting on mature biofilms is additionally assessed, where the critical factor is the capacity to degrade matrix components and thereby increase the accessibility of microbial cells to standard decontamination interventions. The integrated conclusions underscore the fundamental importance of transitioning from universal control schemes to biofilm-oriented validation protocols aimed at reducing residual contamination, enhancing patient safety, and limiting the contribution of the clinical-diagnostic environment to the accelerated spread of antimicrobial resistance.

Keywords: biofilms, microbiological risk, HAIs, sterilization validation, ISO 15883:2024, extracellular polymeric matrix, ATP metry, antimicrobial resistance, medical devices.

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Introduction

During 2024–2025, ensuring the microbiological cleanliness of medical instruments acquired the status of a priority task that determines the resilience and safety of healthcare system functioning. The current global epidemiological situation is accompanied by an increasing share of infections caused by pathogens with multidrug resistance (MDR), for which the ability to form structurally organized and mechanically stable biofilms on abiotic substrates is characteristic [1]. Under these conditions, disinfection and sterilization methods originally optimized for inactivating planktonic microbial forms demonstrate reduced practical effectiveness when encountering mature biofilm conglomerates, in which the protective properties of the matrix and the physiological heterogeneity of the population impede the achievement of complete eradication [3].

The high significance of the problem under consideration is determined by a combination of epidemiological and technological prerequisites. According to World Health Organization data published in 2024, healthcare-associated infections (HAIs) are recorded in a proportion of patients up to 7% in high-development countries and up to 15% in low- and middle-income states; such cases are associated with prolonged inpatient treatment and increased mortality rates, reaching, under unfavorable scenarios, 64.6% [4]. An additional factor is the expanded use of devices that are structurally complex and sensitive to thermal impacts, including contemporary video endoscopic systems and robotic surgical platforms. The presence of multichannel architectures, microcavities, zones with limited accessibility to the flow of cleaning solution, and areas that are difficult to assess visually creates conditions for the emergence of concealed biofilms, the removal of which by standard mechanical techniques becomes practically unattainable [3].

At the 2024–2025 juncture, the need became apparent for a substantial revision of regulatory approaches to medical device reprocessing. The introduction of ISO 15883-1:2024 and ISO 15883-2:2024 establishes updated requirements for the validation of washer-disinfectors, identifying as critical parameters the quality of the final rinse and the reproducibility of thermal disinfection regimens, which is directly linked to controlling residual contamination and preventing re-inoculation of processed surfaces [7].

The aim of the work is to develop and substantiate a biofilm-oriented validation system for the disinfection/sterilization of medical devices that links the effectiveness of pre-cleaning, cycle parameters (including A0 and the quality of the final rinse), and control methods (ATP metry/fluorescence) in order to reduce residual contamination and the risk of HAIs.

The authorial hypothesis is based on the assumption that persistent recurrences of instrument contamination in 2024–2025 are determined not by insufficient sterilization, but by the retention of fragments of the biofilm matrix after pre-cleaning; therefore, the inclusion of a matrix-disrupting enzymatic stage and combined cleanliness control substantially increases the achievable log reduction and the reproducibility of protocols.

Scientific novelty consists in the fact that the study is the first to propose an integral model of evidence-based validation in which the biofilm is treated as an independent object of control (matrix plus heterogeneity), and success criteria are formalized as the sequence removal of matrix → confirmation of cleanliness (fluorescence plus ATP) → confirmation of cycle lethality (A0 / concentration–exposure) → control of the water factor of the final rinse in accordance with ISO 15883:2024/2025.

Materials and Methods

The methodological architecture of the study was constructed on the basis of an integrative analysis of relevant and methodologically robust publications indexed in Scopus and Web of Science, in combination with current and recently updated regulatory acts from the last several years. A systems approach was applied, within which epidemiological analysis, interpretation of experimental data on the effectiveness of disinfection interventions, and comparison of engineering and regulatory requirements for specialized equipment were integrated.

The empirical component relied on official statistical datasets on healthcare-associated infections (HAIs), materials from United States epidemiological surveillance systems (PA-PSRS), and summaries issued by the World Health Organization. For the quantitative characterization of microbiological risks, results from

laboratory tests were used in which processing effectiveness was assessed by the logarithmic reduction metric for clinically significant pathogens, including Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Mycobacterium chimaera.

The adequacy and completeness of validation protocols were evaluated through a comparative analysis of the requirements of the ISO 15883 standards (2024 edition) and ISO 17664, with a focus on critical parameters of thermal disinfection expressed via A0, as well as on approaches to chemical inactivation. In analyzing methods for detecting residual contamination and biological traces, data from prospective studies conducted in 2025 were used, comparing the sensitivity and specificity of ATP bioluminescence and fluorescent labeling as instruments of operational quality control for reprocessing.

The mathematical interpretation of disinfection process effectiveness was carried out through computational models of thermal death time and microbial load reduction coefficients, which ensured a reproducible quantitative assessment of the achieved level of decontamination under different processing regimens. At the discussion stage, a synthetic method was applied, enabling the consolidation of biological, engineering and technological, and regulatory components into a unified risk management framework oriented toward preventing biofilm-associated contamination and enhancing the reliability of validation procedures.

Results and Discussion

In 2024–2025, the epidemiological landscape is characterized by an increase of the contribution of medical instruments and equipment to the maintenance and spread of healthcare-associated infections (HAIs), as reflected in a sustained increase in reported cases and in a shift of the microbiological profile toward biofilm-associated pathogens. Analysis of 2024 data documents unfavorable dynamics within surveillance systems in a number of countries. In the United States, according to information from the Commonwealth of Pennsylvania, the number of reports of infections in long-term care (LTC) facilities increased by 10.6% relative to 2023 and reached 26,501 cases, extending a three-year upward trend that became apparent in 2021 [12].

A structural analysis of the distribution of HAIs by hospital unit type in the Russian Federation demonstrates a concentration of risk in surgical care: surgical departments account for 31.9% of all registered cases [18]. This disproportion is consistent with the high frequency of invasive procedures and the intensive turnover of instruments, which increases the probability of microbial persistence when cleaning effectiveness and subsequent decontamination stages are insufficient. From the microbiological perspective of risk, studies from 2024–2025 indicate dominance of the biofilm phenotype: up to 63% of isolates obtained from patients with HAIs are characterized by a pronounced capacity for biofilm formation [1]. This circumstance is of fundamental significance because biofilm organization increases tolerance to disinfectants, facilitates the survival of viable subpopulations, and promotes chronic contamination on abiotic surfaces [13, 17].

Within the spectrum of leading causative agents, representatives of the gram-negative nosocomial group predominate: Klebsiella pneumoniae accounts for 22%, Pseudomonas aeruginosa for 20%, and Acinetobacter baumannii for 18% [1]. The latter pathogen demonstrates the most pronounced biofilm-forming potential, reaching 72.2%, which allows it to be regarded as a high-priority marker organism for assessing biofilm-associated threats and for selecting target validation regimens for reprocessing medical devices [1].

Table 1 below presents statistics on HAIs and biofilm formation.

Table 1. Summary statistics on HAIs and biofilm formation (prepared by the author on the basis of [1, 5, 18]).

Indicator	Value (2024)	Forecast for 2025
Total number of healthcare-associated infections in the Russian Federation (excluding COVID-19)	19 158 cases (+3,1%)	Expected increase of 2.5–4.0%
Proportion of biofilm-forming strains (global)	63.0%	Stabilization at a level >60%
Case fatality in biofilm-associated infections	7% – 64.6%	Increase driven by multidrug-resistant strains
Mean length of hospital stay (biofilms)	12.4 ± 2.9 days	Persistently above the norm (8.6 days)

The statistical interpretation of the obtained data indicates a pronounced association between the biofilm-forming phenotype and antibiotic resistance, which transforms biofilms into an independent factor driving the selection and fixation of multidrug resistance (MDR). Within a logistic regression framework, it was demonstrated that assignment of an isolate to MDR status is associated with a twelvefold increase in the probability of forming a markedly robust biofilm (OR 12.03) [1]. In this manner, a self-sustaining pathogenetic loop emerges: biofilm organization decreases the vulnerability of bacterial populations to disinfectants, while MDR strains that survive reprocessing, possessing heightened adaptive potential, promote subsequent biofilm formation with even greater tolerance and a predictably increasing difficulty of eradication [21, 22].

A correct appraisal of these risks requires consideration of biofilm architecture and the functional role of its structural components. The extracellular polymeric matrix (EPM), which may constitute up to 90% of the community biomass, serves as a key element of physicochemical protection, functioning as a diffusion barrier to antimicrobial action. Studies from 2024 published in the journal Biology emphasize the significance of extracellular DNA (eDNA) and specialized matrix proteins, which are not limited to mechanically retaining disinfectant molecules: their capacity for electrostatic interaction with active agents and for reducing the effective charge of these agents has been shown, thereby impeding penetration into deeper layers of the biofilm and weakening activity against microbial target structures [19, 23].

Biofilm functional stability is also supported by population heterogeneity, including the presence of persister cells, metabolically quiescent variants that retain viability under impact directed at cell division processes or protein synthesis. Such recalcitrance enables community repopulation even under conditions in which the death of the main cell mass reaches 99.9%, which explains the phenomenon of clinically and technologically meaningful recurrences of contamination after formally successful reprocessing cycles [20]. An additional level of adaptation is reflected in 2025 data showing that exposure to sublethal concentrations of peracetic acid (PAA) can induce, in Klebsiella pneumoniae biofilms, a restructuring of the polysaccharide composition of the matrix, as a result of which resistance to subsequent disinfection cycles increases [3] (see Fig. 1).

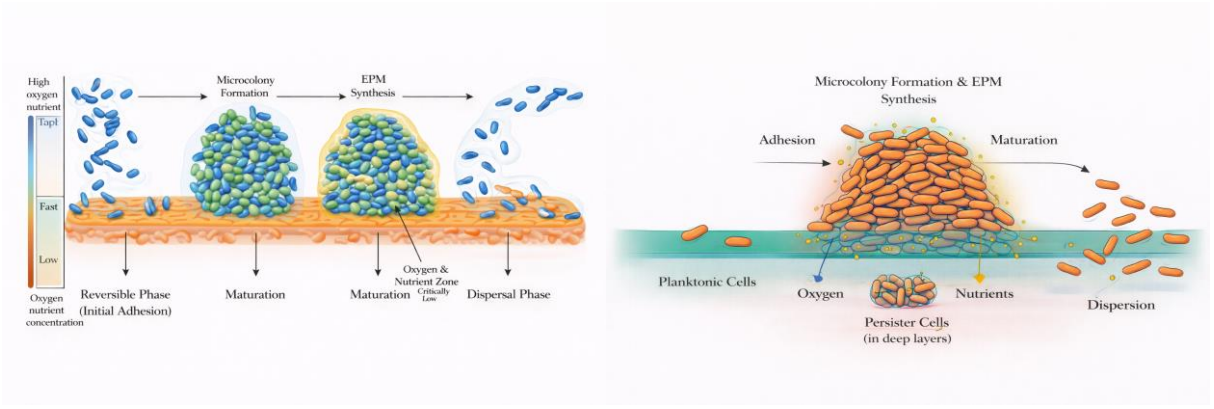

Fig. 1. Stages of biofilm formation on the surface of an instrument (compiled by the author based on [3, 19, 20]).

Peracetic acid (PAA) has traditionally been regarded as the gold standard for high-level disinfection; however, a body of publications updated as of November 2025 indicates limitations of its real-world biocidal performance against biofilm forms of certain strains. In an experimental model using the Bead Assay approach (biofilm formation and cultivation on glass beads), it was shown that a typical PAA concentration of 0.075%, used in automated endoscope reprocessors, does not achieve the threshold reduction of 5 log10 for Klebsiella pneumoniae biofilms [3].

The contrast between the susceptibility of planktonic and biofilm populations within the same strain demonstrates a fundamental difference in the required exposure: at a concentration of 0.001%, killing of planktonic cells was recorded, whereas for a comparable level of effect within

the biofilm, 0.15–0.2% PAA was required [3]. This discrepancy has direct practical significance, as it indicates the likelihood of viable microorganisms persisting in hard-to-access zones, including the internal channels of endoscopes, when protocols oriented toward planktonic forms and nominal concentrations of the active agent are applied [11, 26].

A similar problem has been described for Mycobacterium chimaera, the etiologic agent of severe infectious complications after procedures involving cardiopulmonary bypass devices. Biofilm structures of the M. chimaera strain ZUERICH-1 demonstrated tolerance not only to PAA, but also to standard concentrations of glutaraldehyde, underscoring the systemic nature of biofilm-associated resilience and the need to revise adequacy criteria for high-level disinfection in the context of biofilm contamination models [14].

Table 2 below presents comparative data on the effectiveness of disinfectants against biofilms.

Table 2. Comparative effectiveness of disinfectants against biofilms (prepared by the author on the basis of [3, 14]).

Agent	Concentration (working)	Effect on planktonic forms
Peracetic acid (PAA)	0.075%	> 5 log reduction
Glutaraldehyde (GA)	2.0%	> 4 log reduction
Sodium hypochlorite (Cl)	0.1%	Moderate
Enzymatic cleaners	According to the instructions	Reduction of bioburden

To overcome pronounced biofilm-associated tolerance, an approach was substantiated in 2025 that involves a transition to intensified reprocessing regimens, which, in particular, assume an increase in the concentration of peracetic acid to 0.15% and an extension of exposure to 10 minutes at 37°C.3 At the same time, it is emphasized that such intensification cannot be regarded as a universal solution without prior evidence-based validation, because increasing the concentration of the active agent and modifying temperature–time parameters can affect compatibility with medical device materials, their functional characteristics, and service life [3, 10].

In the context of standardizing thermal reprocessing procedures, the update of ISO 15883-2:2024 is of fundamental significance, as it establishes a contemporary framework of requirements for thermal disinfection. The key calculated indicator remains the A0 parameter, interpreted as the integrated lethality of the process, normalized to an equivalent exposure in seconds at 80°C [24].

For devices classified as critical due to contact with sterile tissues or blood, achieving A0 = 3000 is recommended, which is regarded as a threshold that ensures inactivation of the most thermoresistant pathogens, including hepatitis B virus [24]. For semicritical devices, including, in particular, anesthesia equipment, a target value of A0 = 600 is permissible [25] (see Fig. 2).

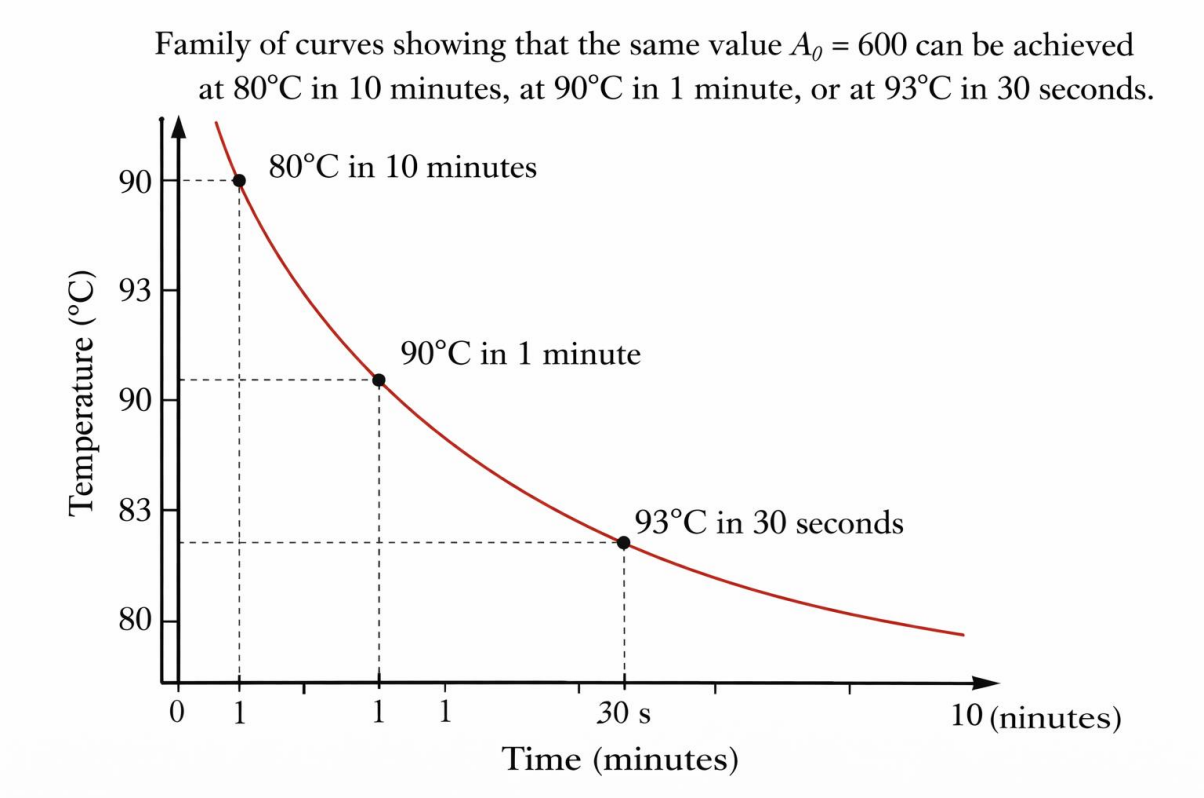

Fig. 2. Graph of the dependence of the A0 value on temperature and time (compiled by the author based on [3, 24, 25]).

The presented Figure 2 reflects a fundamental technological tradeoff inherent to thermal reprocessing: increasing temperature makes it possible to shorten cycle duration, yet simultaneously increases the load on instrument materials and structural elements, thereby raising the probability of polymer degradation, geometric alteration of sensitive components, and accelerated wear under repeated cycling [24].

The update of ISO 15883-1:2024 complements the temperature–time concept of decontamination by strengthening requirements for the water parameters used at the final rinse stage, because this step is capable of negating the achieved level of microbiological safety in the presence of secondary inoculation. It has been shown that faucet aerators in healthcare facilities often function as persistent foci of biofilm formation for Cupriavidus pauculus and Stenotrophomonas maltophilia, thereby creating a risk of recontamination of already processed instruments and equipment [8, 27]. For this reason, the standard establishes a quantitative criterion for the microbial purity of water used for endoscope rinsing at a level of ≤ 10 CFU per 100 mL, which effectively shifts water-factor control from an optional element to a mandatory component of the reprocessing validation system [27].

The evidentiary value of sterilization validation loses practical meaning in the absence of objective confirmation of pre-cleaning quality, because residual organic contamination and biological fluids provide a substrate for microbial retention and accelerate the formation of the biofilm matrix. In 2025, an NHS (United Kingdom) research group presented comparative data evaluating the diagnostic capabilities of ATP bioluminescence and a fluorescence-based approach in assessing the cleanliness of surfaces and instruments [16]. Fluorescence technology, implemented with portable sources of ultraviolet radiation (365 nm) and blue light (450–455 nm), enables rapid visualization of traces of organic matter, pharmaceuticals, and biological fluids that are not discernible under standard lighting, thereby increasing the detection of concealed cleaning defects [16]. A critically important observation was that, in 100% of cases, areas assessed by staff as visually clean demonstrated pronounced fluorescence, indicating residual contamination and underscoring the limitations of visual inspection as an independent acceptability criterion [16].

Table 3 describes the characteristics of cleanliness

control methods.

Table 3. Characteristics of cleanliness control methods (prepared by the author on the basis of [16]).

Parameter	ATP bioluminescence	Fluorescent detection
Control area	10 cm² (point-based)	Up to 40 cm² at a time (screening)
Mean value (success)	33 RLU	Absence of luminescence
Mean value (contamination)	161 RLU	Presence of stains or fingerprints
Consumables	Swabs (costly)	None (after purchasing the flashlight)
Objectivity	Quantitative (RLU value)	Subjective (visual assessment)
Sensitivity to extracellular polymeric matrix	Limited (if few viable cells are present)	High (detects matrix proteins)

The study results demonstrated a statistically significant association (≤ 0.05) between areas identified by a fluorescence signal and elevated ATP bioluminescence values; during assessments of sanitary and plumbing surfaces, levels of up to 1200 RLU were recorded [16]. This convergence indicates that both methods are broadly oriented toward identifying problematic zones where residual organic contamination persists and where conditions for microbial persistence are established.

At the same time, a methodological limitation of ATP metry in the context of biofilms was demonstrated: when inactive or dead bacterial cells predominate within the matrix structure, false-negative results are possible, because signal intensity depends on the presence of metabolically meaningful nucleotides and viable cellular components [9, 16]. In such situations, fluorescence visualization retains diagnostic value because it detects the presence of an organic substrate and elements of the biofilm matrix regardless of the viability of embedded cells [16]. In this context, the importance increases of technological solutions aimed not only at inactivating cells, but, above all, at disrupting the extracellular polymeric matrix (EPM), which restricts diffusion of active substances and reduces the accessibility of microbial cells to standard disinfecting factors.

Because traditional disinfectants exhibit limited penetration into the EPM, a key stage in contemporary biofilm-oriented validation protocols is the use of multicomponent enzymatic cleaners capable of dismantling the matrix into functionally meaningful fragments. A study published in Antimicrobial Agents and Chemotherapy describes the development of the product deconex Prozyme Active, which includes four enzymatic classes: proteases, lipases, amylases, and polysaccharidases [2, 15]. Such a composition provides simultaneous action on protein, lipid, and carbohydrate components of soils and matrix material, conceptually aligning with the requirement for multitarget decontamination of channels and complex cavities.

The effect of the enzymatic approach is reflected in experimental results in which removal of up to 95% of the Staphylococcus aureus biofilm and approximately 90% of the Pseudomonas aeruginosa biofilm was achieved.6 Of fundamental importance is not only the reduction of biomass, but also the decrease in microbial burden: enzymatic treatment led to a 99.23% decrease in CFU (a reduction of 2.11 log), after which subsequent chemical disinfection operated under conditions approximating a planktonic model and ensured a cumulative reduction exceeding 6 log [6]. Thus, enzymes function not as a standalone alternative to disinfectants,

but as a functional amplifier of the protocol, converting biofilm contamination into a more controllable state.

In parallel with biochemical intensification of cleaning, 2024–2025 has seen a strengthening trend toward automation and digitalization of reprocessing as tools for reducing variability and eliminating human-factor effects. By 2024, the market volume of automated washer-disinfectors (WD) was estimated at 1.24 billion United States dollars, and the forecast for 2033 projects growth to 2.18 billion, reflecting a systemic demand for standardizability, reproducibility, and complete traceability of critical operations [28]. In practical implementation, such complexes are increasingly integrated with hospital information systems, while device identification and traceability are ensured through RFID tags, enabling cycle parameters to be documented at the level of a specific instrument and a specific load [29].

At the same time, digital transformation introduces new vulnerabilities associated with software reliability and the correctness of data transmission. An FDA analysis published in November 2024 showed that, among 950 medical devices with artificial intelligence components (AIMDs), 60 were recalled, with 43% of recalls occurring during the first year of operation; key causes included software errors and delays in data exchange [30]. These findings underscore that digital validation cannot be limited to confirmation of physical cycle parameters (temperature, pressure, time, rinse quality), but must also include verification of algorithmic logic, data integrity, and the correctness of interpretation within the decision-making loop.

Taken together, the presented data reflect a clear shift in the scientific and practical logic of ensuring microbiological safety. The priority characteristic of twentieth-century approaches, oriented primarily toward inactivation of single, predominantly planktonic microbial cells, in the conditions of 2024–2025 yields to a paradigm in which the central object of control becomes biofilm bioarchitecture. Within this framework, the critical concern is not so much the probability of survival of an individual bacterium as the persistence of a structurally and functionally viable fragment of a biofilm. Even a limited residual conglomerate that preserves matrix elements and a viable micropopulation can serve as a source of a rapidly initiated and persistence-prone infectious process, for which increased tolerance to antibiotic therapy and reduced accessibility to innate and adaptive immune factors are typical. Validation of reprocessing protocols, therefore, must be grounded in recognition of a collective survival mechanism, in which the protective properties of the matrix, population heterogeneity, and spatial isolation of microzones determine the ultimate effectiveness of deactivation to a greater extent than the nominal activity of the disinfecting agent.

Conclusion

The conducted analysis makes it possible to formulate several fundamental conclusions that define the current state and development directions of validation for disinfection and sterilization protocols in 2024–2025.

The traditional planktonic paradigm of disinfectant testing in clinical practice has lost explanatory and predictive adequacy. The availability of experimental evidence demonstrating that standard concentrations of peracetic acid are insufficient to achieve required reduction levels for Klebsiella pneumoniae biofilms indicates the need to revise operational algorithms for reprocessing endoscopic equipment and to update regulations that provide for increasing the concentration of the active agent or applying combined interventions oriented toward the biofilm organization of contamination.

The implementation of the updated ISO 15883:2024/2025 standards establishes a qualitatively new level of requirements for equipment manufacturers, service organizations, and the infrastructure of central sterile services departments. The strengthened emphasis on reproducibility of thermal parameters, including achievement of A0 = 3000 for critical devices, and on the microbiological safety of final-rinse water transfers the relevant indicators into the category of mandatory and regularly confirmed parameters. Validation in this logic ceases to be limited to checking the functional operability of the washer-disinfector and expands into systemic control of the water circuit, including laboratory assessment of water quality and identification of conditions that promote biofilm formation in elements of the hospital water supply system.

Quality control of pre-cleaning is justifiably regarded as a key predictor of the effectiveness of subsequent decontamination stages, which necessitates a transition to a two-level monitoring scheme. In the 2025 practical model, the combination of fluorescence-based rapid control, applicable for rapid assessment of large surfaces

and for strengthening procedural discipline, with quantitative ATP metry used as an instrumentally confirmed criterion during formal validation of the most significant cleaning stages, is recognized as rational.16 Exclusive reliance on visual inspection in these conditions loses the status of an acceptable control method and should be qualified as noncompliance with safety requirements, because it does not ensure detection of concealed organic residues and matrix-associated contamination.

The chemical and technological strategy of pre-sterilization cleaning under biofilm-associated risks should be reoriented toward the use of multicomponent enzymatic systems that ensure disruption of polysaccharide and protein components of the matrix. In such a framework, the enzymatic stage becomes a critical condition for achieving the required lethality of subsequent interventions, because it is precisely the destruction of the EPM that increases accessibility of microbial cells to chemical disinfection and reduces the likelihood of preserving viable microreservoirs.

Automation and digital integration of reprocessing processes, despite the limitations identified in 2024–2025 related to the reliability of software components and the need to verify algorithms, appear to be a strategically inevitable development direction. The transition to closed technological loops with digital identification and traceability of reprocessing, implying the creation of an electronic passport for each device, provides a foundation for evolution from selective checks to continuous monitoring of quality and safety at the level of each load and each manipulation.

Overall, the presented positions and practice-oriented recommendations are aimed at reducing microbiological risks and require incorporation into existing infection control programs of healthcare organizations, because only the integration of biofilm-oriented validation principles, objective monitoring methods, and systemic standardization can ensure consistently high levels of patient safety under contemporary conditions.

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