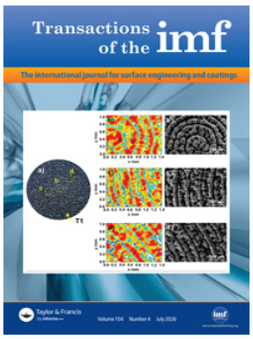

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BULLETIN

International standards for evaluating antibacterial, antiviral, and antibiofilm properties of surface-finished materials: a critical review

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ABSTRACT

International standards such as ISO 22196, ISO 21702, and ISO 4768:2023 provide essential frameworks for evaluating the antibacterial, antiviral, and antibiofilm properties of plastics and other non-porous surfaces. Although these standards have greatly contributed to reproducible performance assessment, they remain primarily endpoint-based biological assays and do not fully integrate the physicochemical characteristics of engineered material surfaces. This critical review compares the scientific basis, experimental assumptions, and practical limitations of the three standards from a surface engineering perspective. Particular attention is given to the film-contact principle, crystal violet staining, metal ion dissolution, wettability, surface roughness, intermolecular forces, and extracellular polymeric substances (EPS). The discussion encompasses representative biofilm-forming organisms – including *Pseudomonas aeruginosa* and *Staphylococcus aureus*, fungal species such as *Candida albicans* and *Rhodotorula mucilaginosa*, and industrially relevant *Bacillus* and *Micrococcus* species – whose behaviour on engineered surfaces underpins the rationale for each standard. The review argues that antibacterial, antiviral, and antibiofilm evaluations should not be treated as isolated test categories, because all are governed by common surface – microorganism interactions. At the same time, important gaps remain, including the lack of standardised flow-based antibiofilm testing, insufficient treatment of mixed-species biofilms, and limited requirements for surface characterisation. Future standardisation should therefore move beyond pass/fail biological activity values and incorporate materials descriptors, real-time monitoring, and predictive approaches such as electrochemical impedance spectroscopy, image analysis, and digital twin modelling. Such integration will strengthen the scientific basis of anti-infective surface design and improve the reliability of performance claims for practical applications.

KEYWORDS

Antibacterial; antiviral; antibiofilm; ISO 22196; ISO 21702; ISO 4768; biofilm; surface engineering

1. Introduction

The COVID-19 pandemic brought renewed scrutiny to the role of material surfaces in microbial transmission[1,2], prompting both public awareness and regulatory interest in the anti-infective performance of engineered materials. In response, manufacturers, standardisation bodies, and researchers have accelerated the development and harmonisation of evaluation methods for antibacterial, antiviral, and antibiofilm properties. Three international standards now form the principal framework: ISO 22196 for antibacterial activity[3], ISO 21702 for antiviral activity[4], and ISO 4768:2023 for antibiofilm performance[5]. Although each standard addresses a distinct class of microorganism, they share a common foundation in surface – microorganism interactions governed by physicochemical forces such as wettability, intermolecular attraction, and metal ion dissolution[6]. This review examines these three standards from a surface engineering perspective, offering a comparative analysis that highlights both their scientific basis and their practical limitations for materials development.

2. Antimicrobial evaluation: ISO 22196 and the film covering method

2.1. The film covering method and quantitative evaluation

ISO 22196:2011[3] specifies a quantitative method for evaluating the antibacterial activity of plastics and other non-porous surfaces. The test is based on the film covering (or film contact) method, in which a standardised bacterial inoculum is placed on the specimen surface and held in intimate contact by a covering film during incubation (Figure 1). This configuration ensures reproducible contact between the test organism and the material surface, distinguishing it from alternative approaches summarised in Table 1[7].

The film covering method is illustrated schematically in Figure 1. A standardised bacterial inoculum is placed on the specimen surface and covered with a non-reactive polymer film (e.g. polypropylene) to ensure intimate contact. The assembly is incubated at 35 °C under humidity below 90% for a prescribed period. After incubation, the surviving organisms are recovered, inoculated onto agar medium, and incubated at 35 °C for 24 h; the resulting colonies (Figure 2) are enumerated as colony-forming units (CFU).

Antibacterial activity, A, is defined by Equation (1):

$$A = (\log C_{24} - \log C_0) - (\log T_{24} - \log T_0) = F - G \quad (1)$$

where $F = \log C_{24} - \log C_0$ is the increase in viable cell count on the untreated control over the 24-hour incubation

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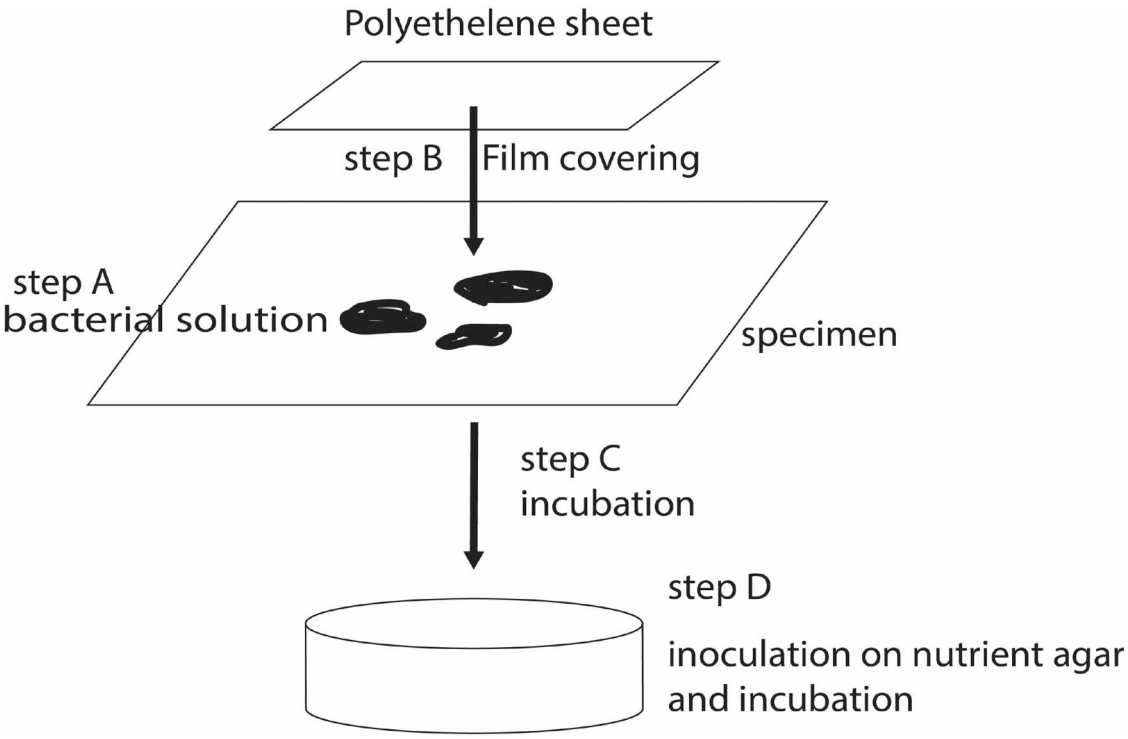

Figure 1. The schematic procedure for antibacterial material's evaluation and film covering method.

period, and $G = \log T_{24} - \log T_0$ is the corresponding increase on the test specimen; C_0 and T_0 are the viable cell counts (CFU) on the untreated control and the test specimen, respectively, immediately after inoculation, and C_{24} and T_{24} are the corresponding viable cell counts after the prescribed 24-hour incubation period. If $\log C_0 > \log T_0$, the value of $\log T_0$ is replaced by $\log C_0$ in the calculation. A value of $A \geq 2$, corresponding to a 99% reduction in viable bacteria, is taken as the threshold for antimicrobial activity.

The film contact geometry is central to the reliability of the method, as it ensures a reproducible interface between the bacterial suspension and the material surface. However, the covering film itself is not entirely inert: variations in adhesion between the film and the specimen can influence the effective contact area and, consequently, the test outcome. The alternative methods summarised in Table 1 – the drop, shake, dip, and halo methods – were developed to address specific limitations of the film covering approach [7,8]. The drop method eliminates film-related artefacts but introduces variability in droplet spreading and risks bacterial desiccation. The shake and dip methods accommodate non-flat specimen geometries at the cost of reduced surface specificity. The halo method provides only semi-quantitative assessment, dependent on the diffusion characteristics of the active agent. Selection among these methods requires consideration of specimen geometry, the mechanism of antimicrobial action, and the acceptable level of quantitative precision[9].

Table 1. Comparison of antimicrobial test methods applicable to surface-finished materials.

Method	Principle	Surface geometry	Key Standard(s)	Limitation
Film covering (film contact)	Inoculum pressed against surface by polymer film	Flat, non-porous	ISO 22196; JIS Z 2801	Limited to flat specimens
Drop method	Inoculum droplet placed directly on surface	Flat	N/A	Evaporation; poor contact control
Shake (flask) method	Specimen immersed in bacterial suspension with agitation	Any geometry	ASTM E2149	Dilution effect; not surface-specific
Dip method	Specimen dipped into bacterial suspension	Any geometry	N/A	Short contact time
Halo (zone of inhibition) method	Specimen placed on inoculated agar	Flat	ISO 20645; JIS L 1902	Semi-quantitative; diffusion-dependent

2.2. Limitations of ISO 22196 from a surface engineering perspective

Despite its widespread adoption, ISO 22196 carries several limitations that materials scientists should recognise when interpreting reported activity values. The most fundamental is its restricted treatment of the time axis. The 24-hour endpoint captures only the initial bactericidal response of a fresh surface against a single inoculation event, and the static film-contact configuration excludes the dynamic conditions encountered in service – repeated wetting and drying, shear-induced removal of conditioning films, and progressive depletion of surface-bound active agents. As a consequence, an activity value of $A \geq 2$ certifies short-term efficacy but provides no information on the durability of the antibacterial effect under repeated or prolonged challenge, which is the primary concern in real-world applications such as touch surfaces, medical devices, and water-contacting infrastructure.

Two further limitations compound this temporal narrowness. First, the standard tests a single bacterial species in mono-culture (commonly *Escherichia coli* and *Staphylococcus aureus*), whereas industrial and clinical surfaces are colonised by mixed communities whose synergistic resistance

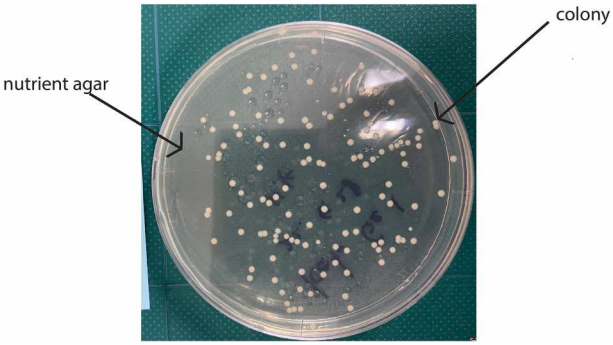

Figure 2. Observation of colonies on nutrient agar.

mechanisms develop over longer timescales and are absent from the assay. Second, ISO 22196 does not require reporting of the physicochemical descriptors that govern the interactions it is intended to evaluate – surface roughness (R*a*, Rz), water contact angle, surface energy, and, for metal-ion-releasing coatings, the ion release rate at the test temperature and humidity. Without these descriptors, the activity value cannot be mechanistically interpreted, nor extrapolated to surfaces of comparable but unmeasured composition.

These limitations do not diminish the value of ISO 22196 as a reproducible screening tool. Rather, they define the boundaries within which its results should be interpreted and motivate the extension of evaluation along the time axis – first towards viral inactivation kinetics in ISO 21702, and ultimately towards the long-term sessile communities addressed by ISO 4768, which is examined in the following sections.

3. Antiviral evaluation: ISO 21702

3.1. Test methodology and antiviral activity value

ISO 21702:2019[4]specifies a method for measuring the antiviral activity of plastics and other non-porous surfaces. The test protocol shares the same film contact geometry as ISO 22196: a viral inoculum is placed on the specimen surface under a covering film and incubated for a defined period, after which the surviving infectious virus is recovered and quantified. Two principal quantification approaches are employed. In the $TCID_{50}$ (50% tissue culture infectious dose) assay, serial dilutions of the recovered virus are inoculated onto host cell monolayers, and the dilution at which 50% of the cultures exhibit a cytopathic effect is determined statistically[10,11]. Figure 3 shows the two principal quantification approaches employed in ISO 21702. In the plaque assay, the recovered virus is overlaid onto host cells under semi-solid medium, and discrete plaques formed by localised cell lysis are counted directly[11]. The antiviral activity value is calculated as the difference in logarithmic titre between an untreated reference surface and the test specimen.

A critical distinction from the antibacterial standard lies in the biological complexity of the target: whereas bacteria are free-living organisms capable of independent metabolism, viruses require host cells for replication (Figure 4). Consequently, ISO 21702 evaluates the capacity of a surface to inactivate viral particles – that is, to render them unable to infect host cells – rather than to inhibit metabolic growth. This difference has direct implications for the surface engineering strategy: antiviral efficacy depends on disruption of the viral envelope or capsid through surface chemistry (e.g. metal ion release from copper or zinc coatings), photocatalytic generation of reactive oxygen species, or physicochemical destabilisation via controlled wettability[12,13].

3.2. Limitations of ISO 21702 from a surface engineering perspective

ISO 21702 shares both the strengths and the same narrow time window as ISO 22196: the same film-contact set-up, the same static incubation, and the same short fixed endpoint. The most pressing problem for materials evaluation is that the standard cannot tell us how quickly a surface inactivates a virus; it only reports the result at one fixed time. Yet studies during and after the COVID-19 pandemic showed that infectious SARS-CoV-2 and related coronaviruses can survive on plastic, stainless steel, and glass for hours to days [1,2]. How fast they lose infectivity depends strongly on temperature, humidity, and the chemistry of the surface. A single-point activity value tells us nothing about this decay rate, so it cannot rank surfaces by *how quickly* they reduce viral load – which is the very property that matters most for stopping infection through contaminated surfaces (so-called *fomite* transmission).

Three further limitations follow. First, the test usually uses only one virus, typically influenza A or feline calicivirus. But enveloped and non-enveloped viruses can behave very differently on the same surface – sometimes by a factor of a thousand or more – because breaking the lipid envelope plays a much bigger role in killing the former than the latter. Second, the standard does not specify the lighting conditions during incubation. This is a serious gap for photocatalytic coatings such as titanium dioxide or zinc oxide, whose antiviral action depends on UV or visible light to generate reactive oxygen species. Third, like ISO 22196, ISO 21702 does not require the user to report the surface descriptors that govern the very interactions it is trying to evaluate – wettability, roughness, and (for metal-bearing coatings) ion release rate.

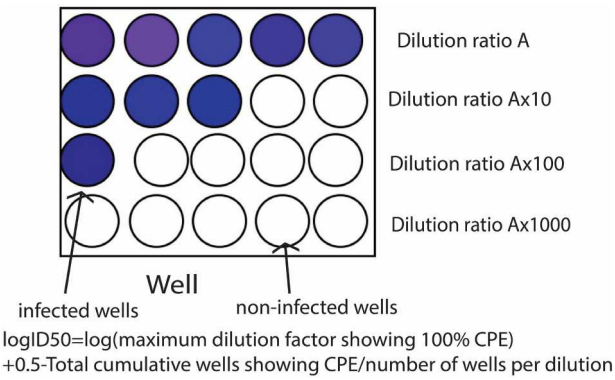

Figure 3. Schematic illustration of the $TCID_{50}$ assay.

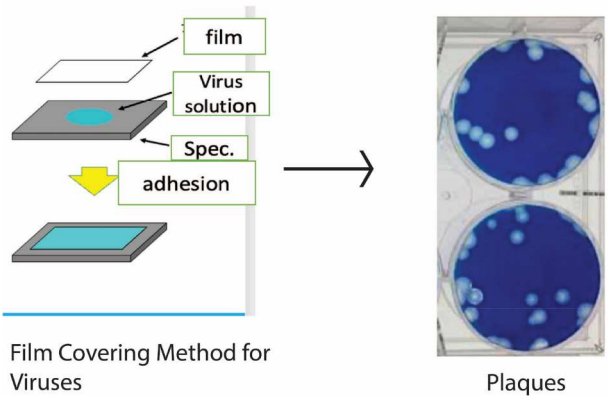

Figure 4. Plaque assay of viruses and the film covering method.

Taken together with the limitations of ISO 22196, these gaps reveal a wider pattern. The two standards evaluate how surfaces respond to *individual* microbial entities – free-swimming bacteria and isolated virus particles – over short, static incubation periods. They tell us little about the long-term, dynamic interactions between surfaces and microbial *communities*. It was precisely this gap that motivated the development of ISO 4768:2023, which addresses biofilms – the longest-lasting and most complex form of microbial settlement on surfaces – and which is examined in the following section.

4. Antibiofilm Evaluation: ISO 4768:2023

4.1. Biofilm formation mechanisms and the role of EPS

Figure 5 shows the canonical sequence of biofilm development. Biofilm formation proceeds through a well-characterised sequence: reversible attachment of planktonic cells to a conditioned surface, irreversible adhesion mediated by extracellular polymeric substances (EPS), micro-colony development, maturation into a three-dimensional architecture permeated by water channels, and eventual dispersal [14–17]. The EPS matrix – comprising polysaccharides, proteins, extracellular DNA, and lipids – serves both as a structural scaffold and as a barrier that limits the penetration of biocides and host immune factors[14].

Among bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are widely regarded as model biofilm-forming organisms owing to their clinical relevance and robust EPS production[18,19]. Species of *Bacillus* and *Micrococcus* are also recognised for their propensity to colonise industrial surfaces, particularly in water distribution and cooling systems. Fungal biofilms, notably those formed by *Candida albicans* and *Rhodotorula mucilaginosa*, present additional challenges because of the morphological transition between yeast and hyphal forms, which enhances structural complexity and antifungal resistance[20].

Environmental temperature exerts a significant influence on biofilm development. Mesophilic organisms such as *P. aeruginosa* exhibit optimal biofilm formation between 25 and 37 °C, whereas psychrotrophic species can establish biofilms on refrigerated infrastructure. ISO 4768:2023 specifies incubation at 25 ± 1 °C for 48 h using a static reactor system, a condition selected to accommodate a broad range of industrially relevant organisms while maintaining reproducibility across laboratories.

4.2. Laboratory biofilm reactors: static vs. flow systems

A central challenge in standardising antibiofilm evaluation is the selection of an appropriate laboratory reactor. Biofilm reactors fall broadly into two categories: static systems, in which the test specimen is immersed in a quiescent nutrient medium, and flow systems, in which fresh medium is continuously or intermittently supplied across the specimen surface.

Static systems, exemplified by the microtitre plate assay and the modified Robbins device operated in batch mode[21,22], offer simplicity, low cost, and high throughput. Their

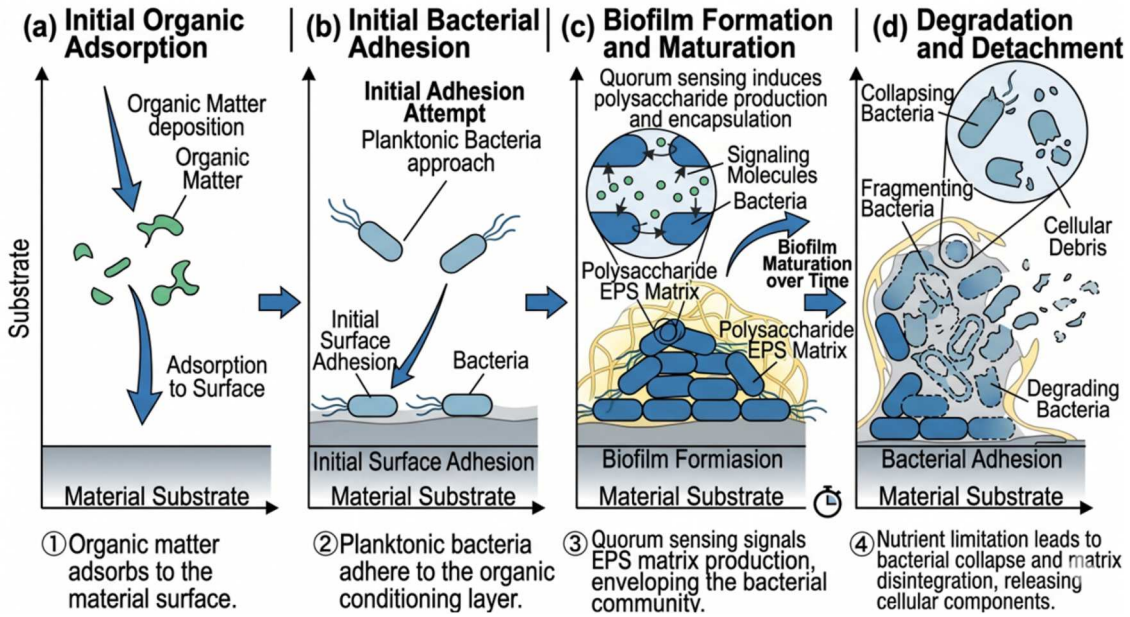

Figure 5. Schematic illustration of Biofilm Formation Process

principal limitation is the absence of hydrodynamic shear, which governs biofilm morphology and mechanical resilience under service conditions. ISO 4768:2023 adopts a static reactor configuration using a six-well plate format, in which test coupons are submerged in a bacterial suspension and incubated without agitation. This choice reflects a pragmatic compromise: static conditions maximise inter-laboratory reproducibility while remaining accessible to testing facilities without specialised flow equipment.

Flow systems – including the CDC biofilm reactor (ASTM E2562)[23], drip-flow reactor (ASTM E2647)[24], and rotating disc reactor[25] – provide controlled shear stress and continuous nutrient renewal, yielding biofilms whose structure more closely resembles that encountered in pipelines, heat exchangers, and medical devices. However, the additional variables (flow rate, residence time, reactor geometry) reduce reproducibility and complicate standardisation. The authors' experience in establishing ISO 4768 confirmed that consensus among international delegates favoured the static approach as a necessary first step, with flow-based extensions anticipated in future revisions of the standard.

4.3. Crystal violet staining and quantitative evaluation

ISO 4768:2023 prescribes crystal violet (CV) staining as the primary method[5] for quantifying biofilm formation on test specimens (Figure 6). The procedure involves three stages: (i) gentle rinsing of the coupon to remove loosely attached planktonic cells, (ii) staining of the adherent biofilm with an aqueous CV solution (typically 0.1% w/v) for a defined period, and (iii) solubilisation of the bound dye in ethanol or acetic acid, followed by absorbance measurement at 590 nm. The antibiofilm activity value is then calculated as the percentage reduction in absorbance of the test specimen relative to the untreated reference surface, in analogy with the antibacterial activity value defined in ISO 22196 (Figure 7).

CV staining offers several practical advantages: it is inexpensive, requires only a spectrophotometer, and stains the entire EPS matrix – polysaccharides, proteins, and extracellular DNA – rather than viable cells alone. This characteristic is significant from a surface engineering standpoint, because industrial biofilm problems (e.g. microbially influenced corrosion, biofouling of heat exchangers[6,26]) are driven by the presence of the EPS matrix itself, irrespective of whether the constituent cells remain viable.

The method does, however, carry recognised limitations. CV is a non-specific dye that cannot distinguish between living and dead biomass, nor between bacterial and abiotic organic deposits. Variability in rinsing intensity can alter the retained biofilm mass, and the staining efficiency may differ among EPS compositions. For these reasons, complementary techniques – confocal laser scanning microscopy (CLSM) with live/dead fluorescent staining[27], colony-forming unit (CFU) enumeration, and dry-weight gravimetry – are recommended in the literature as confirmatory measures, although they fall outside the mandatory scope of ISO 4768:2023.

4.4. Comparison with ASTM and EN biofilm standards

Although ISO 4768:2023 is the first international standard dedicated to antibiofilm evaluation of material surfaces, several related standards and test protocols exist within the ASTM and EN frameworks[28–30]. Table 2 summarises the principal methods and their distinguishing features.

A fundamental distinction lies in the objective of each standard. ASTM E2562 and E2647 characterise biofilm *growth* under defined hydrodynamic conditions but do not prescribe a method for evaluating the *anti*biofilm performance of a material. ASTM E2799 addresses the efficacy of antimicrobial agents applied *to* a pre-formed biofilm, rather than the intrinsic resistance of the material surface to biofilm formation. EN 13697 and EN ISO 846 assess biocidal or biodegradation behaviour, which overlaps with but is not equivalent to antibiofilm activity as defined by ISO 4768.

ISO 4768:2023 thus occupies a unique position: it evaluates the capacity of the material surface itself to suppress biofilm formation under standardised conditions, providing a single quantitative metric (antibiofilm activity value) that is directly comparable across materials and laboratories. The first author, who participated in the drafting process through the SIAA biofilm committee, note that achieving international consensus on this scope required deliberate narrowing of the test conditions – static incubation, CV staining, and a 48-hour endpoint – to ensure accessibility and reproducibility as a first-generation standard.

4.5. Limitations and the path towards next-generation antibiofilm standards

ISO 4768:2023 represents an important step forward – it is the first international standard to evaluate the *long-term* response of surfaces to microbial communities, rather than the short-term response to individual microbes. Even so, the standard inherits some of the limitations identified in Sections 2.2 and 3.2, and adds a few of its own that the surface engineering community is well placed to address.

The first limitation is the choice of static incubation. The 48-hour endpoint is longer than the 24-hour endpoints of ISO 22196 and ISO 21702, but the test still does not include flow. Real biofilms in pipelines, heat exchangers, marine structures, and medical devices form under continuous or intermittent flow, and the resulting wall shear stress strongly affects biofilm thickness, density, and mechanical strength. A flow-based extension – based on reactors such as the CDC biofilm reactor (ASTM E2562)[23], the drip-flow reactor (ASTM E2647)[24], or the rotating disc reactor[25] – should therefore be considered as a future addition to the standard. Computational fluid dynamics analysis can support such an extension by mapping the wall shear stress distribution inside candidate reactors and guiding coupon placement[31].

The second limitation is the use of mono-culture testing. ISO 4768 specifies *Staphylococcus epidermidis* as the test organism, selected in part for its biosafety level 1 (BSL-1) status. Real industrial and clinical biofilms are almost always mixed-species communities[32,33], in which inter-species cooperation can produce synergistic resistance that is invisible to a single-species test. Future revisions of the standard should at least allow, and ideally encourage, the use of defined multi-species consortia.

The third limitation lies in the quantification method itself. Crystal violet (CV) staining is robust and inexpensive, but it stains all biomass

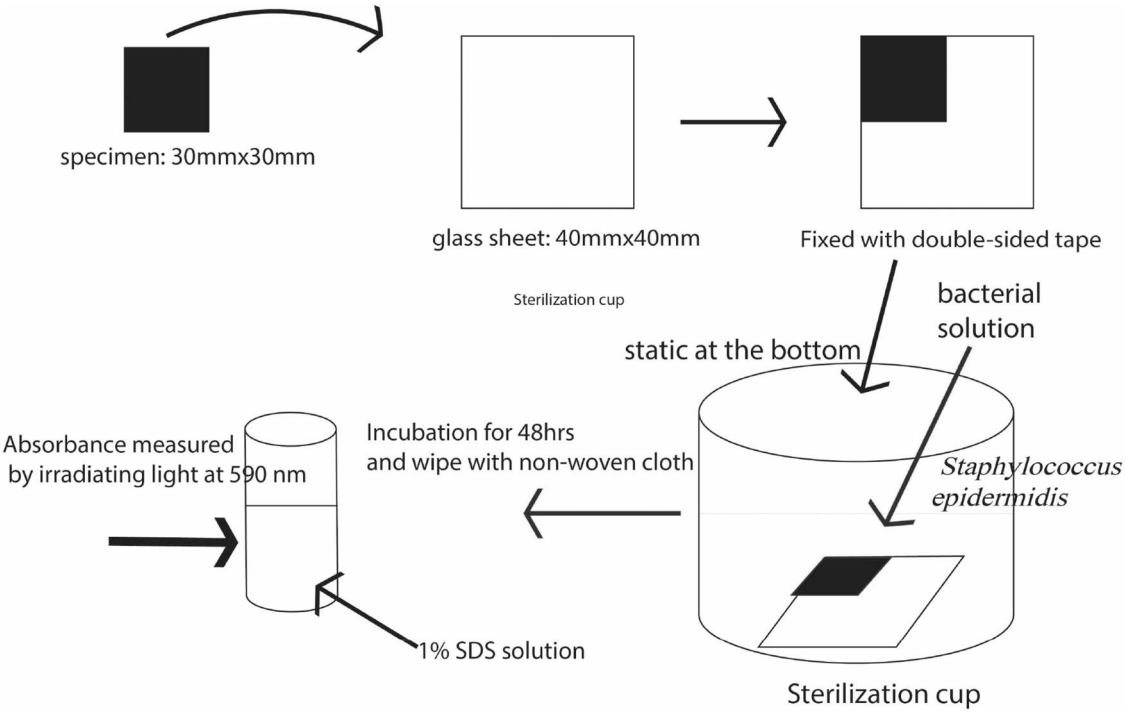

Figure 6. Process of biofilms evaluation based on crystal violet staining.

without distinguishing between live cells, dead cells, and abiotic deposits. It also gives only one number per coupon: a measure of biofilm *amount*, not biofilm *structure*. Yet the structure of a biofilm – its thickness profile, its porosity, its degree of clustering – often determines its resistance to cleaning and disinfection more than its total mass does. Confocal laser scanning microscopy (CLSM) with live/dead staining[27] can provide three-dimensional structural information, and quantitative image analysis applied to such data can extract structural descriptors – biofilm thickness, porosity, and degree of clustering – that complement the CV-based activity value.

Finally, ISO 4768, like ISO 22196 and ISO 21702 before it, does not yet require the reporting of surface descriptors – roughness, wettability, surface energy, ion release rate – that materials scientists know to be central to biofilm formation. Without these descriptors, antibiofilm activity values cannot easily be compared between studies or extrapolated to new materials.

None of these points should be read as a criticism of ISO 4768 in its current form. Rather, they identify the directions in which a second-generation antibiofilm standard might evolve. Three developments seem particularly promising. First, the integration of *real-time, non-destructive monitoring* – for example using electrochemical impedance spectroscopy (EIS)[34] or quartz crystal microbalance with dissipation monitoring (QCM-D)[35] – would replace the single-endpoint activity value with a continuous record of biofilm growth and removal. Second, the integration of *quantitative three-dimensional image analysis* would let the standard capture biofilm structure rather than just biofilm mass. Third, *digital twin modelling*[31] of biofilm reactors and of the surfaces they contain could, in due course, allow virtual evaluation of new materials before any laboratory test is performed. Together, these developments point towards a unified framework in which materials descriptors, real-time monitoring, and predictive modelling work together to support the rational design of anti-infective surfaces – a framework whose physicochemical foundations are examined in the next section.

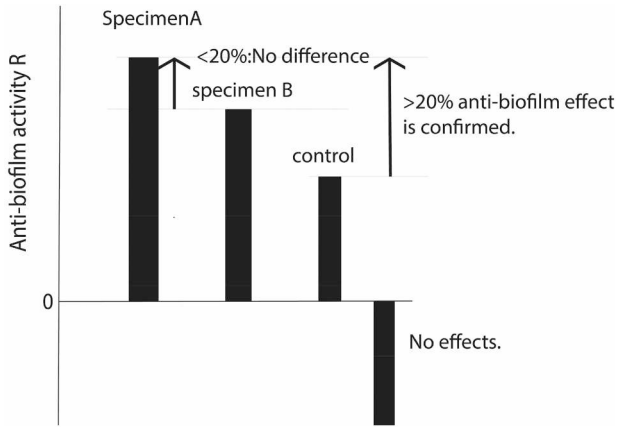

Figure 7. Antibiofilm activities and the interpretation example.

5. Comparative discussion: common principles across the three standards

The three standards reviewed in this paper address distinct biological targets – planktonic bacteria, viral particles, and sessile biofilm communities – yet the underlying surface – microorganism interactions are governed by a common set of physicochemical principles. This section presents a unified framework (illustrated schematically in Figure 8) that links the anti-infective performance of a material surface to three interrelated mechanisms: (i) metal ion dissolution and its biocidal or EPS-disrupting action, (ii)

Table 2. Comparison of biofilm evaluation standards and methods.

Standard / Method	Scope	Reactor type	Quantification	Organism(s) specified
ISO 4768:2023	Antibiofilm activity of non-porous surfaces	Static (six-well plate)	CV staining (A_{590})	*Staphylococcus epidermidis* (ATCC 35984,BSL-1)
ASTM E2562	Biofilm growth under moderate shear	CDC biofilm reactor (flow)	CFU, dry weight, or CV	User-defined
ASTM E2647	Biofilm growth under low shear	CDC biofilm reactor (flow)	CFU, dry weight, or CV	User-defined
ASTM E2799	Efficacy of antimicrobial agents against biofilm	MBEC assay (static, peg lid)	CFU after challenge	User-defined
EN 13697	Bactericidal / yeasticidal activity of surfaces	B Carrier test (static)	CFU reduction	Specified panel
EN ISO 846	Resistance of plastics to microorganisms	Static immersion / burial	Visual and mass change	Fungi and bacteria

intermolecular forces at the surface – microorganism interface, and (iii) surface wettability as characterised by water contact angle.

5.1 Metal ion dissolution and surface chemistry

The release of metal ions from coatings and surface treatments constitutes the most widely exploited antimicrobial mechanism in surface engineering. Copper and silver ions disrupt cellular respiration and membrane integrity in bacteria (ISO 22196), destabilise viral envelopes and capsid proteins (ISO 21702), and interfere with EPS cross-linking in nascent biofilms (ISO 4768)[36]. Zinc ions, incorporated into surface finishes through zinc-rich primers or nano-zeolite dispersions, exhibit broadly similar behaviour[37]. The critical parameter from a materials design standpoint is the ion release rate, which must be sustained above the minimum inhibitory concentration (MIC) at the surface – liquid interface throughout the service life. Electrochemical techniques – polarisation curves and electrochemical impedance spectroscopy – offer a means of predicting long-term dissolution kinetics and correlating them with the activity values defined in each standard[34].

5.2. Intermolecular forces at the interface

Initial attachment of bacteria, viruses, and EPS precursors to a surface is mediated by a balance of van der Waals attraction, electrostatic (Coulombic) interaction, and acid – base (Lewis) forces, collectively described by the extended DLVO theory[38]. For antibacterial and antibiofilm performance, surfaces that minimise attractive interactions – for example, through low-surface-energy fluoropolymer coatings[39] or hydrophilic poly(ethylene glycol) brushes[40] – reduce the probability of irreversible adhesion. For antiviral performance, the situation may be inverted: surfaces engineered to attract and retain viral particles can concentrate them locally, thereby enhancing contact-mediated inactivation by co-present biocidal agents. This duality underscores the importance of evaluating anti-infective properties across all three standards when developing multifunctional surface treatments.

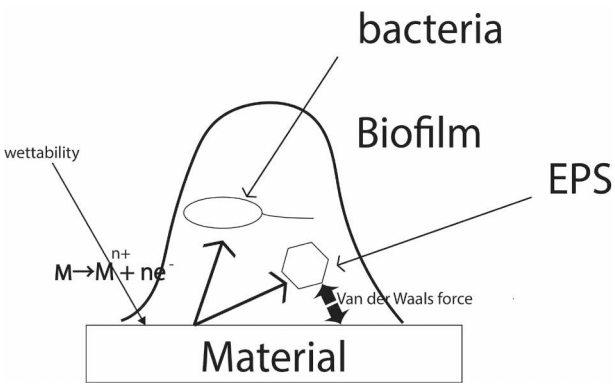

Figure 8. Mutual interaction between materials and biofilm.

5.3. Wettability and contact angle

Water contact angle serves as a convenient macroscopic descriptor of surface energy and is routinely measured during quality control of coated and plated products. Highly hydrophilic surfaces (contact angle < 30°) tend to form a stable water film that can either dilute bacterial adhesins and viral receptors or, conversely, promote wetting of crevices in which biofilms preferentially nucleate. Highly hydrophobic surfaces (contact angle > 110°) reduce aqueous contact area and can impede bacterial spreading, yet may adsorb proteins and conditioning-film components that subsequently promote biofilm maturation. The consensus emerging from recent literature is that extreme wettability in either direction is less effective than a moderately hydrophilic surface (contact angle 40° – 70°) combined with controlled surface topography[41–43]. This observation has direct relevance to surface finishing processes – electroplating, anodising, chemical conversion coatings, and organic film deposition – all of which allow systematic tuning of both wettability and roughness.

Taken together, these three mechanisms define a design space within which surface engineers can optimise anti-infective performance against bacteria, viruses, and biofilms simultaneously. The unified framework proposed here suggests that future revisions of each ISO standard would benefit from requiring characterisation of surface energy and ion release rate alongside the biological activity value, thereby strengthening the link between materials science and microbiological evaluation.

6. Implications for surface engineering and future directions

The three ISO standards examined in this review collectively define the current benchmark for evaluating the anti-infective performance of materials, yet they also expose gaps that the surface engineering community is uniquely positioned to address.

6.1. Advanced antimicrobial and antibiofilm coatings

Significant progress has been made in translating laboratory antimicrobial

concepts into functional surface finishes. Silver-containing coatings – deposited by electroplating, sputtering, or incorporation into organic matrices – remain the most commercially mature approach, with efficacy demonstrated across all three standards.[36,44] Copper and copper-alloy surfaces have attracted renewed interest following evidence of rapid contact-mediated inactivation of SARS-CoV-2[12,13,45]. More recently, nano-zeolite dispersions loaded with silver or zinc ions have emerged as a versatile platform[37,46,47]: the zeolite framework acts as an ion-exchange reservoir, sustaining controlled release over extended periods and enabling incorporation into electrodeposited, sol – gel, and UV-curable resin coatings.

Beyond metal-ion-based strategies, biologically active polymers incorporating quaternary ammonium compounds[48], chitosan derivatives[49], or polyphenol moieties[50] offer a complementary route to anti-infective surfaces. Polyphenols such as catechin and epigallocatechin gallate (EGCG) interfere with bacterial quorum sensing and suppress EPS production, thereby targeting the biofilm phenotype specifically. Photocatalytic coatings based on titanium dioxide[51] or zinc oxide[52] generate reactive oxygen species (ROS) under UV or visible-light irradiation, providing a self-renewing biocidal mechanism applicable to both antibacterial and antiviral contexts. These emerging technologies share a common challenge: validation against internationally recognised standards. The framework of ISO 22196, ISO 21702, and ISO 4768 provides the necessary reference, but testing protocols may require adaptation – for example, light-activated coatings necessitate controlled irradiation during the incubation period, a condition not currently prescribed in any of the three standards.

In a related context, the positional isomerism of benzoic acid derivatives has been shown to influence antibacterial activity significantly, with the relative position of hydroxyl and carboxyl substituents governing both membrane disruption potency and selectivity among Gram-positive and Gram-negative species[53]. Such structure – activity relationships underscore the need for standardised evaluation protocols capable of discriminating between subtle differences in surface-bound antimicrobial chemistries.

6.2. Towards real-time evaluation and digital integration

Current evaluation methods are inherently endpoint assays: specimens are incubated for a prescribed duration, after which surviving organisms or biomass are quantified destructively. This approach, while essential for standardisation, cannot capture the dynamic kinetics of microbial attachment, growth, and inactivation that determine real-world performance.

Electrochemical impedance spectroscopy (EIS)[34] and quartz crystal microbalance with dissipation monitoring (QCM-D)[35] offer the potential for non-destructive, real-time tracking of biofilm accumulation on coated surfaces. Integration of such sensor data with computational models – including digital twin frameworks[31] and three-dimensional image analysis of biofilm morphology – could enable predictive assessment of anti-infective service life, moving beyond the pass/fail logic of current standards towards performance-based material design.

6.3. Standardisation needs

Several areas warrant attention in future revisions of the existing standards or in the development of new ones. First, the absence of a standardised flow-based antibiofilm reactor limits the applicability of ISO 4768 to quiescent environments; extension to defined shear conditions would broaden its relevance to pipelines, marine structures, and medical devices. Second, none of the three standards currently addresses mixed-species biofilms, which predominate in natural and industrial settings[32,33] and exhibit synergistic resistance mechanisms absent in mono-culture assays. Third, harmonisation of surface characterisation requirements – mandating the reporting of surface roughness, water contact angle, and elemental composition – would strengthen correlations between materials properties and biological performance, accelerating the rational design of next-generation anti-infective surfaces.

7. Conclusions

This review has set out the case that ISO 22196, ISO 21702, and ISO 4768:2023 should be read not as three independent test methods, but as a single, evolving framework for evaluating how engineered surfaces interact with microbial life. Reading them in this way exposes both the achievements of the existing standards and the directions in which they need to grow.

The most important common thread is the *time axis*. ISO 22196 captures the bactericidal response of a surface in 24 h; ISO 21702 captures viral inactivation over a similarly short window; ISO 4768 extends the observation period to 48 h, but still under static conditions. Each step lengthens the time scale and broadens the biological target – from planktonic cells, to free virions, to sessile communities – yet none of the three standards captures the long-term, dynamic, mixed-species reality of surfaces in service. Recognising this progression makes it clear that the next generation of anti-infective standards will not be built by adding a fourth standalone document, but by extending the existing framework along the same axis: longer observation, more realistic flow, more representative communities, and richer surface characterisation.

For the surface engineering community, the practical implication is direct. An $A \geq 2$ result on ISO 22196, an antiviral activity value on ISO 21702, or an antibiofilm activity value on ISO 4768 should be reported alongside – not in isolation from – the surface descriptors that materials scientists already measure as a matter of routine: roughness (R*a*, R*z*), water contact angle, surface energy, elemental composition, and, for ion-releasing coatings, ion release rate under the test conditions. Reporting these descriptors together with the biological activity value would, at very little additional cost, transform the existing standards into a usable bridge between materials data and antimicrobial performance.

Looking further ahead, four developments will determine whether the standards keep pace with the materials they are meant to evaluate: (i) the addition of flow-based antibiofilm protocols, supported by computational fluid dynamics analysis of candidate reactors[31]; (ii) the controlled introduction of defined multi-species consortia in place of single-organism testing; (iii) the embedding of mandatory surface characterisation requirements in the

reporting clauses of all three standards; and (iv) the gradual integration of real-time, non-destructive monitoring – for example by electrochemical impedance spectroscopy[34] and quartz crystal microbalance with dissipation monitoring[35] – together with predictive frameworks such as digital twin modelling[31], which would in time allow new surface treatments to be screened before any biological test is performed. Achieving these developments will require sustained dialogue between materials scientists, microbiologists, and standardisation bodies. The surface finishing community, with its long experience of relating microstructure and surface chemistry to in-service performance, is well placed to lead this dialogue rather than simply respond to it.

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Declaration of generative AI use

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