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**GUIDANCE DOCUMENT ON USE AND DEVELOPMENT OF TIER-2
LABORATORY BASED TESTS USED TO SUBSTANTIATE CLAIMS FOR
EFFICACY OF BIOCIDES TREATED ARTICLES
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**GUIDANCE DOCUMENT ON USE AND DEVELOPMENT OF TIER-2 LABORATORY BASED TESTS USED
TO SUBSTANTIATE CLAIMS FOR EFFICACY OF BIOCIDES TREATED ARTICLES**

IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among **FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD**

Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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Foreword

The work on developing guidance for evaluating antibacterial activity of biocide treated materials started in 2008 within the then Task Force on Biocides (TFB) now Working Group on Biocides (WGB). An Expert Group on Biocides Treated Articles (EBTA) was formed, and the project included in the work plan of the Test Guidelines Programme in January 2011.

Subsequently a Guidance Document for a Tier-1 Quantitative Method for Evaluating Antibacterial Activity of Porous and non-Porous Antibacterial Treated Materials ENV/JM/MONO(2014)18 was published in July 2014.

The current document aims to extend the abovementioned Tier-1 guidance, on how to assess the efficacy of treated articles, with Tier-2 laboratory based testing. The below document divides treated articles into two major groups (preserving the functions of the article itself / introducing new properties), and provides example protocols for the second function (deposition by aerosols and splashes / deposition by hand contact).

This Guidance Document was approved by the Working Group of the National Coordinators of the Test Guidelines Programme (WNT) at its 30th meeting in April 2018. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 30 June, 2018.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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TIER 2 EFFICACY ASSESSMENT OF TREATED ARTICLES

Summary

1. The range of treated articles and treated materials produced is vast. However, they all have one property in common in that they are intended to express some sort of function against biological systems in service. Articles treated with biocides can be divided in two major groups: one where the biocide preserves the functions of the article itself, the other where new properties are introduced by the biocidal treatment.

2. In the first major group, the function is intended to protect the material or article itself from deterioration or to maintain its properties. It may be intended that the service life or durability is increased or enhanced. Certain claims may be made and expectations about performance of the biocide introduced. In all of these cases it is important that such uses and claims be supported by demonstrating what happens if such a material or article is not treated. The treatment should be shown to be necessary or of sufficient value that the benefit from its use can be demonstrated. The use of the treatment is intended to maintain the properties and value of the material or article to which it has been applied, whether in terms of economic or environmental impact (it lasts longer, does not need replacing etc.). When this is clear, a more accurate judgement of risk vs. benefit can be made for the active substances involved. Fortunately, there are a wide range of test methodologies that can be used (or modified) to demonstrate this need. Furthermore, when applied in combination with ageing and pre-treatments, they can be used to show the benefit introduced. The one area that at present cannot be modelled with ease is the prevention of odour in garments and shoes. Data from wearing trials and the like must therefore be used to demonstrate benefits (although some laboratory-based tests can be used to add supporting evidence).

3. The other major group are materials or articles that are treated to introduce new properties. The most common of these are related to perceived hygienic benefits. These range from preventing the growth of bacteria that may affect the health of people and livestock to articles with properties intending to kill organisms that come into contact with them. The claims made for such effects are diverse and range from the highly specific ('these pyjamas are treated to remove MRSA from the skin prior to admission to hospital') to the very vague ('antibacterial'). To understand the effects that these types of materials and articles are intended to have, requires a clear explanation of what the problem is and how the use of the article will reduce or eliminate it. For example, if a material is treated to prevent growth then it must be shown that growth does indeed occur under normal conditions of use and that the treatment prevents this. It should be made clear why this is of benefit. Articles that are intended to kill organisms that come into contact with them should be tested to show that this is what they do and that this happens under the normal conditions of use, and when the target species are introduced into the test system in the manner in which they would be in practice. The speed, scale and duration of the effect should be shown to be sufficient to deliver the benefit claimed (if a door handle is treated to reduce the spread of nosocomial infections it must be shown that this effect is achieved when relevant organisms are presented through skin contact - simulated or otherwise - and the effect is of sufficient speed and size that it can produce a benefit in

normal use). Although there are a large number of methods that can detect basic antimicrobial properties in such materials, none are suitable without, often, extensive modification, to demonstrate effects in practice. Therefore data that support claims must be generated using tests that are tailor-made to either specific or function-related claims (i.e. tests that reproduce the conditions under which growth/survival could occur, that present the target species in a way that simulates skin contact or is delivered via aerosols and droplets etc.).

4. Regulatory decisions cannot be made based on claim alone. The intent behind the treatment is a fundamental issue that must be understood. Only by demonstrating the reason why a treatment is required, how it is achieved and what benefit it delivers can valid assessments be made of risk vs. benefit for both active substances used in treated articles and treated materials as well as the articles and materials themselves.

Introduction

5. The purpose of this document is to provide guidance on the efficacy testing of biocides used in materials and treated articles. It aims to help define the problem that the use of a biocide is intended to solve or the property that it is intended to introduce and why that is required. This, along with service expectations, will help define the type of data that would be required to support any claim made in a meaningful way. The presence and relevance of existing standard test methods are described and, where they do not exist or where they do not provide sufficient support, the nature of the data required will be described. Two example protocols for common claims are provided.

6. This document provides a structure for possible claims for treated articles and treated materials. It provides, through examples and background information, guidance on different types of claims and how to support them by providing data that is relevant to actual use. At the same time, it raises awareness of the limitations of certain testing regimes in supporting specific claims. The document is not exhaustive but intended to help with the understanding of the underlying principles for formulating the benefits of a biocidal treatment of a material or article. This is a prerequisite for risk vs. benefit judgements.

Understanding the Problem, and the Purpose of a Biocidal Treatment

7. One of the fundamental pre-requisites for understanding the role that a biocidal active substance / product must fulfil is an understanding of the problem that their use is intended to either resolve or, at least, mitigate. The deterioration of natural products such as wood and wool through the action of biological agents, whether microorganisms or insects etc., is familiar and has been associated with them since they were first used by mankind (Selby, 1966). Similarly, there has been a long history of the use of chemical and physical measures to prevent their biodeterioration. With other, especially man-made products such as plastics, the need for protection against biological activity is often less obvious for the end-user outside of the industry involved in their manufacture, distribution and sale. In many cases, the general public is unaware that biocides are even incorporated into them. Even when a biocidal treatment is openly promoted, claims for the antimicrobial (especially antibacterial) properties of treated articles and materials are often unclear and poorly defined. In many cases end-users and consumers have little understanding of the benefit that can be expected and whether it is delivered by the final product in actual use. For example, when one purchases a hard surface disinfectant, one expects that it will kill microorganisms on the surface that is treated with it. However, when one purchases a dishwashing sponge that claims to be antibacterial it is far

from clear what it is intended to do. Does it mean that surfaces wiped with it are disinfected? Is it killing bacteria while you wash the dishes or does it mean that bacteria will not grow on the sponge when it is left moist beside the sink? And for how long will it continue to function for? Many such products lack clarity of purpose.

8. With all biologically active substances there is a balance to be made between any negative aspects of their properties to man and the environment and the benefits that their use may bring. In some cases the presence of a biocide will enhance or extend the performance of a material whereas in others it will be essential to achieve that performance in the first place. For example, many of the current water-based formulations could not be manufactured without an in-can preservative. In the past, the presence of solvents etc. rendered the products non-susceptible to microbiological attack. A water-based paint, free of volatile organic compounds (VOCs), could not exist without the use of a biocide. Likewise, many materials manufactured from plasticised PVC would soon become fouled by surface growths of fungi and lose plasticity and crack without the inclusion of a fungicide (Pantke, 1977). In these examples the biocide is adding a vital function to the material / article. For others, the value that the presence of a biocide brings is in extending their durability, as with facade coatings that have been equipped with a dry-film preservative. In a similar way, the functionality of a material may be enhanced by the presence of a biocide. For example, by treating the textile from which a tent is manufactured, it can be packed and stored after use even if still damp without the risk of the development of mildew. In other examples, new properties may have been added through the addition of biocidal active substances, creating surfaces with enhanced hygienic properties etc.

The Risk Benefit Equation

9. The choice of biocide will depend on its compatibility with the material to be treated and the processes involved in the manufacture of the material and the final article. Which biocide to choose and at what concentration will also depend on the scale, speed and type of effect required to achieve the benefit envisaged. The description of this and the demonstration of it in a manner that simulates at least some of the range of end-uses and final treated articles is important. It provides the counterpoint necessary when making risk vs. benefit assessments of biocidal active substances and products.

10. The end use and performance required affect not just the concentration of active substance(s), but also the way they interact with their surroundings (emissions to the environment, skin, food, the possibility of resistance being developed to an active substance or, more significantly, cross resistance with active substances used for clinical purposes etc.) and are important for judging the risk vs. benefit balance. A vital set of questions is therefore what type of activity is required and how much and for how long? One of the purposes of this document is to help identify what the data that is required to answer these questions should look like and how it should be generated.

The Difficulty with Setting Performance Criteria

11. With certain biocidal products such as disinfectants, there are many national and regional performance criteria that are employed to determine how effective a product needs to be under a certain set of conditions. The approach is often based on suspension tests and the performance in practice may differ from that shown in such tests. For example, disinfectant wipes are often tested for efficacy by expressing the liquid from them and using that in a suspension test (e.g. containing 8 ml product plus 1 ml of a soiling agent and 1 ml of a bacterial suspension). Similarly, products applied by trigger spray or by aerosol are often tested in the

same way and not by simulating their use. Even when they are then applied to a dried deposit of bacteria on a test plaque (as in EN13697 - BS EN, 2015; OECD, 2013), the volume of product to surface area is dramatically larger than would be employed in practice. Also, the methods do not include the mechanical effects of wiping (and consequent physical removal / re-deposition) or any foaming mechanisms etc., although an attempt has been made to address this in EN 16615:2015 (EN, 2015). Despite this, there is wide acceptance that, in most cases, the tests provide a useful measure and can be related to expectations in practice. In contrast, for the majority of treated materials, whether the treatment is intended to protect the material / article in service or to introduce hygienic or disinfectant-like properties into it, there is little clear relationship between concentration, performance under a set of test conditions and performance in use. Indeed, the scale of an effect may differ quite dramatically for a material depending on the detail of its formulation, the properties of the active substance(s), the conditions under which it will be deployed and the life-span it is expected to achieve.

12. As an example, a surface coating equipped with a fungicide to protect the finished film from the growth of moulds (see Figure 1) when used in humid interior environments (e.g. in bathrooms) will probably require a lower level of protection than a similar coating used on an external facade, where exposure to rain and UV radiation will have an impact on the retention and stability of some active substances. A biocide with a relatively high solubility in water would likely be capable of providing protection to a coating intended for interior use but would probably be less suited for use outdoors and maybe be lost rapidly from the film. Even if this were not the case, the concentration required to achieve adequate performance for an exterior application would almost certainly need to be significantly higher than for an interior one. Thus, in even a simple application, it would be difficult to set an arbitrary performance criterion no matter what testing regime were employed.

Figure 1. Mould Growth on a Painted Surface



13. Many plastic formulations are susceptible to growth and spoilage by microorganisms (Wessel, 1964). For example, fungi can utilise the plasticisers incorporated into certain formulations of polyvinyl chloride (PVC) as a source of nutrients. The depletion that results causes a loss of plasticity and the polymer becomes brittle and cracks as well as becoming

visibly fouled by the growth (see Figure 2, which shows both growth as well as partial erosion of the substrate and some physical changes). Polyurethane, for example, as used for the soles of shoes, can, through normal use, become colonised by fungi and actinomycetes. The subsequent growth results in discolouration (often a deep purple in colour) and structural failure. The nature of these two polymer systems requires the use of biocidal products with different physical and chemical properties to be used to prevent premature loss of function in use. These differences in properties have an impact on the suitability of a test method to predict performance. A plastic (e.g. plasticised PVC) equipped with a fungicide with moderate water solubility could be shown to demonstrate fungistatic properties using an agar overlay technique such as described in ISO 16869: 2008 (Appendix A). This test relies on sufficient active substance migrating from the plastic into the medium placed over it to inhibit the germination of the fungal spores contained in it. If this occurs it would be expected that the treatment would provide protection to a finished article in service. The use of a campaign of pre-leaching etc. could even be employed to help provide data on anticipated service life. However, another active substance with a much lower solubility in water, may fail to demonstrate activity using the same standard. Even though it may provide sufficient protection in practice, it may not produce a sufficiently high enough concentration of active substance in the overlay to inhibit the growth of fungi in the test medium. So not only are performance criteria dependent on the end use conditions, but some testing procedures are more suitable for detecting activity and predicting performance of certain active substances than others. Making comparisons based on one type of test alone can be highly misleading. This does not mean that one method superior to another but that the method must be suited to the active substance, material and end use. Making comparative assessments of different active substances and substrate based on a single protocol is impossible in most cases.

14. In some application areas there is a wide diversity of standard tests available to measure the performance of treated materials whereas in others there are few, if any. With some materials, for example masterbatches¹ of plastics, the same treated material might be used to manufacture a wide range of products intended for use in a wide range of end applications. The demands on their performance will likely be highly diverse. This makes the task of assessing claims and making judgements about risk vs. benefit even more difficult. The purpose of this document is to provide guidance for this difficult process.

¹ A masterbatch is a carrier material or plastic additive into which pigments and/or other additives (such as a biocide) have been dispersed. It is often comprised of granules or pellets of the same material as the main plastic into which it will be added at a specified concentration prior to blending and extrusion / moulding. In many ways a masterbatch can be considered to be analogous with a formulated biocide. In the latter, one or more active substances are combined with other components to provide a formulation that can be used to treat a material / article to either to provide protection or to introduce new properties and functions. Similarly, a masterbatch is a blend of a polymeric matrix and one or more active substances. It is added to a polymer blend just before it is used to create a moulded or extruded final product. It may impart either protection to the finished article or introduce new properties and functions.

Figure 2. Microbial Growth on a Plasticised PVC Shower Curtain

The Role of Biocides in Materials and Articles²

15. **Overview:** The purpose of this section is to identify broad sub-divisions in the reasons why biocides are used to treat materials and articles. It describes the division of such uses into

² Whether a treated material falls under either the treated articles definition of the European Biocidal Products Regulation (BPR) or within the scope of the definitions used by the US EPA, Canada etc is not considered in this document. Although this is important for the question of whether an article has to undergo a national authorization procedure, it is not decisive for the testing of its efficacy.

The claim being made (or the intent implied) will define in which of the Product Types (PTs) described in Annex V of the Biocidal Products Regulation (BPR) an application will fall. In some legislations, such as the US and Canada, they either require that the whole article be registered using an agreed protocol or that it be registered with a different agency. In an example where the primary function of a treatment is to protect a material the application will usually fall under a PT within Main Group 2 in the EU BPR. However, if the intentions of the treatments are effects related to health, this would require that the active substance used was registered within Product Types 1 - 4 of Main Group 1. In the US this would result in the article itself requiring registration and in Canada the article would fall under the jurisdiction of the Therapeutic Products Directorate (TPD). Claims under PT 18 or 19 of Main Group 3 of the EU BPR might be either intended to protect material, e.g. a woollen carpet or health related, e.g. mosquito-repelling clothes. Claims related to both types of benefit would require supporting data, but health related claims usually require further justification, evidence and proof. For example, carpets and bedding materials are often treated to inhibit odour and to prevent the growth of moulds. The consequence of this effect might result in a claim that through doing this, sensitive individuals are less likely to have allergic responses due to the presence of fungal spores. This is sometimes extended to suggest that because mould growth is inhibited, the presence of mites, which feed on the fungi, will be reduced and the resultant reduction in frass will reduce further any allergic response. Clearly such claims would require an extensive demonstration that such a sequence of effects does take place under normal conditions of use and that the treatment reduces it.

either protecting the properties of materials and articles from damage by biological action (i.e. the primary role is the protection of materials) or introducing new, often health-associated, properties into a material (i.e. the primary role is protection of humans and livestock). When biocides are incorporated into materials or used in the production of treated articles they tend to be used with two purposes in mind. They are added either to:

- protect the materials used in the article or the properties of the article in service or,
- to introduce a new property to an article that is not related to the primary function of the article. These claims are usually health-related. Please note that in some legislations health related claims alter both the way the product is regulated and the body responsible for their registration.

16. For example, plasticised PVC is protected from damage by fungal growth by the inclusion of a biocide; a suitable service life for an exterior surface coating is ensured through the use of a film-protective biocide formulation. However, a biocide applied to a dish-washing sponge has not been included to protect the material used, but to introduce some sort of self-disinfecting or hygienic property to the article. In some instances this rather broad categorisation may seem to overlap and materials that are protected from damage through microbiological attack are perceived as being more hygienic because they end up, for example, free of mould growth. Of course, one is the consequence of the other but, in this instance, the primary purpose of the biocide is to protect the material. This differentiation is vital to understanding the role of a biocide and the effect it is intended to deliver.

Demonstrating that a Biocide is Required

17. Whether a biocide is included in a material / article to either protect it or add new properties to it, a clear benefit should result. It must be possible to demonstrate the reason that the biocide has been incorporated and any claim made should be both meaningful and realistic. When used to protect a material / article, it is important to be able to demonstrate that the material / article would be either damaged, lose essential properties or function or be compromised in terms of durability in service if the biocide were not present. If the biocide has been incorporated to add new, disinfection-like or other health related properties to a material or article, these should be demonstrated to function under the conditions in which it will be used and be capable of delivering a meaningful benefit. In some regions, focus has been placed on claims that relate to public health; products and articles that do not make such claims (providing the active substance is registered) are exempt from registration as biocidal products (providing they do not employ certain trigger phrases etc. US EPA, 2000). This has resulted in a large number of products being placed onto the market that claim that they contain a biocide to protect the product from microorganisms even though they would be unaffected by them in the first place (e.g. ‘this pen has been equipped with a biocide to protect it from bacteria’). The approach may have prevented the use of unsubstantiated claims but has failed to prevent the unnecessary use of biocides, as a demonstration of the benefit of their use has been omitted from the equation.

18. Despite the fact that there will inevitably be some overlaps and applications that require a decision to be made, a general division has been made in this document. Effects related to the integrity of the material or article or its function are considered in the following two sections: “Protection of a material/article and its properties” and “Prevention of odour”. Those that are intended to introduce new properties that are not related to its primary function are subsequently considered in a separate section. The purpose is to provide a structure to help understand what benefit is being introduced and how it should be substantiated.

Protection of a Material / Article and its Properties

19. **Overview:** The purpose of this section is to examine claims related to the protection of materials and articles and their properties as well as the extension of their service life. Typical claims associated with these materials and articles include: ‘treated to inhibit the growth of mould’, ‘mould resistant’, ‘mildew resistant’, ‘protected from the growth of fungi and algae’, etc.

20. The protection of wood is, in general, not considered in this document because a lot of guidance exists already, although claims for wood / plastic composites may fall within its scope.

21. There are many methods capable of describing the effects claimed and, with care, these can be employed to support claims. A selection of the more common methods, sorted by material, is listed in Appendix A.

22. The function of many biocidal products is obvious and their performance is relatively straightforward to demonstrate. For example, a wet state preservative used in paint and other water-based formulations is intended to protect the product during manufacture and prevent spoilage until the product is used by the consumer. Without such protection the product would quickly become colonised by microorganisms. The metabolism and growth of these would cause undesirable changes to it, such as the development of foul odours, loss of stability (as dispersants and the like are destroyed) and function. The choice of the type of preservative and its concentration etc. is based on technical and commercial considerations, and some form of wet-state challenge test (Lunenburg-Duindam and Lindner, 2000) is employed (often along with storage phases) to test the efficacy. For active substances, simpler protocols using a relevant model substrate can be employed (Gillatt et al., 2015). The same principle can be applied to other treated materials in which the properties introduced are intended to be manifested in service and that are related to the protection of the material, its properties or its function (e.g. metal cutting lubricoolants).

23. Although the properties that the inclusion of a biocide in a material / article might introduce may overlap, it is possible to divide the effects they produce into three broad subdivisions. This process will help in the understanding of the type of demonstration that is required to support the claim for the benefit intended.

Protecting the Properties of a Material

24. Many materials are susceptible to damage by organisms. In some instances this damage is readily visible (and it may be that the visual appearance is what needs to be protected). In others it is the mechanical properties of the material that are affected. In many cases, a combination of adverse effects is observed. Facade coatings intended to improve the aesthetics of a building can become fouled by the growth of fungi and algae. Plastics in which additives have been incorporated to make them highly flexible become brittle when fungi biodegrade the plasticiser. PVC shower curtains are treated with a fungicide to prevent both staining and discolouration and to protect the plasticiser (see Plate 2). As with an in-can preservative, the role of the biocide and the benefit of the treatment is clear and indeed vital to maintaining the properties and function of the treated material. With materials such as PVC, polyurethane, non-synthetic textiles, wood-plastic composites etc. the need for the inclusion of a biocide in the material can be readily demonstrated either by studying the behaviour of untreated materials under conditions of normal use or in an accelerated test in the laboratory. The addition of a

biocide adds little extra value to the product as it is intended to preserve essential properties of the material / article.

Extending Service Life and Durability

25. A major application for many biocides is to increase the service life of a material or an article. In many cases, this increase is related to the protection of the actual material itself and is thus an extension of the use described in the section above. Many materials are not susceptible to biological deterioration when freshly manufactured but, upon entering service, begin to change (either chemically or physically) and become susceptible. They may also become soiled in service and the presence of soiling may result in colonisation with organisms, the growth of which may cause incidental damage to the material supporting them. Similarly, the aesthetic appearance of a material may be a vital component of its function and the growth of disfiguring organisms would lead to the loss of this function and result in increased maintenance or premature replacement. For example, a house decorated with a facade coating that is equipped with a film fungicide and algicide will not require redecorating due to the presence of fungal and algal growth as frequently as one that is not. The presence of the biocides extends the durability of the appearance and integrity of the protective film. Powder coatings and coil coatings for rolled sheet metals are sometimes similarly equipped both to preserve their physical appearance (as they cannot be redecorated in situ) and to reduce slip hazards that result from microbiological growth on their surface (e.g. steel ladders and industrial walk-ways). There are clearly economic benefits from the inclusion of the film protection and probably environmental ones (impact of producing more paint etc.) as well as benefits related to function and safety.

Figure 3. Fungal and Algal Growth on a Façade



Protecting the Function

26. In some materials, a biocide may be added to extend its functional properties even where the material itself is not susceptible to biodeterioration under the normal conditions of use. For example, the silicone-based materials used to manufacture insulating sleeves in exterior high-voltage applications are, generally, not susceptible to damage by the growth of microorganisms. Fungal growth may cause staining on such materials but rarely results in loss of their functional properties under normal use and changes to the aesthetic properties are not usually a major consideration. In service, such sleeves do support the growth of fungi and algae on their surface and the presence of this changes moisture retention and affects the insulating properties of them. This results in arcing and short-circuits leading to interruptions in the supply of electricity. To combat this, and sustain the function of the insulating sleeve, biocides with fungicidal and algicidal properties are incorporated into the polymer system. Similarly, biocides can be added to the coating systems used to produce retro-reflective road signs etc., where algal and fungal growth cause changes to their reflective properties, making them harder to see after dark. So, although the materials themselves are not vulnerable to the growth of microorganisms, the final articles or their functions are. Even with these rather indirect applications though, the effect required is well understood and can be observed or measured. It can also be modelled such that the impact of the inclusion of a biocide can be evaluated and a cost-effective addition can be determined.

Determining the Level of Performance Required

27. Determining the performance of biocides intended to preserve the function and integrity of materials shown in the examples above is a relatively straightforward and intuitive process. There are a number of standard test protocols (see Appendix A) that can be employed for such purposes and the consequences of failure are plain to see and can be measured in both functional as well as commercial terms. Simulating the service life is often achievable and will usually employ a cost - benefit analysis by the end user of the biocide (i.e. the manufacturer of the material / article). In most instances, the choice of biocide and its concentration will be determined by the in-service expectation and the impact that its inclusion has on the economics of its production. The failure of a treated material or article to live up to the claim made for it will result in the failure of the product in service, leading to complaints (and possibly legal action) up the supply chain and, at the very least, will mean a loss of a repeat sale (if one bought a shower curtain that went mouldy after a few months in service one might complain and expect a replacement or one might simply avoid that brand in the future). The impact of failure would be observed in the market in some form or another.

28. An essential principle for the claim that a biocide protects the properties or function of a material or article, or that it increases their durability is to show that when it is absent, these properties, functions or its durability are compromised. However, although a manufacturer may observe that their product becomes contaminated in practice, it can sometimes be difficult to reproduce this completely under laboratory conditions. This may be due to a lack of suitable test strains and an inability to reproduce the factors that influence the product during manufacture and in use due to the various interactions involved. Therefore, when producing data to be used in support of either active substance or product registration, it is often necessary to use a model test matrix which can be shown to be susceptible to a set of relevant test species rather than a real, final matrix (for example, a specific polymer dispersion formulation). In this way the effect of the biocide can be demonstrated in principle for certain families of final

products. In practice, some biocidal products will be better suited to some formulations than others and a manufacturer will select the one best suited to achieve the degree of protection required in practice for their final article. Similarly, the concentrations required in a model test matrix will often be lower than those required in practice, as factors such as ageing (durability, shelf life etc) and decreased stability due to chemical and physical interactions are often not represented in such model systems. In true final products, with a specified shelf-life, this can be simulated through appropriate periods of storage, maybe at elevated temperatures or soiling. Thus, through a combination of laboratory-based simulations and knowledge of the requirements of final products, a solution to a range of actual problems can be described. This would enable an evaluation of the benefit that a particular biocidal active substance / product can deliver. Maximum use rates can be estimated and routes of emission described such that risk vs. benefit can be evaluated.

Demonstrating the Need and Measuring the Effect

29. One of the most important factors in any risk / benefit analysis is to understand why a biocide is required to protect a material or to improve its performance. Thus, when producing data that demonstrates this, it is important to use a matrix, even if it is a model one, to show the damage phenomenon and how the inclusion of the biocide affects it. There are many methods available worldwide that are used to study the effect of microorganisms (and other biological systems) on materials (a list of some of the more common ones is given in Appendix A). On the whole, biological tests for studying biodeterioration tend to fall into two basic types (simulation tests, see §30-32 and laboratory tests based on artificial growth media, see §33-35). Different factors trigger the choice of a test: in some cases the choice of one type of method over another is related to the speed with which it generates results. Often, a method is 'known' to be capable of guiding the choice and concentration of a biocide for a certain material through experience within an industry. Simulation and laboratory tests will be considered separately although it should be noted that one type of method is not necessarily superior to another even when one appears to simulate the end use better than another. In some cases it can be very difficult to demonstrate effects in the laboratory, even in a simulation test, and data from a field trial must be employed. For example, there are few robust methods for assessing the impact of treated materials on the generation of odours in garments and footwear. This is discussed separately below and the role that laboratory data can play in assessing such claims is explored.

Simulation Tests

30. The ideal test method would present a material to a consortium of relevant test organisms under conditions that simulate realistically those that it would encounter in real life. This would produce effects that are identical to those observed in practice and allow a treatment to be identified with precision. There are methods that come closer to this ideal than others. For example, BS 3900 Part G6 (Appendix A) exposes painted panels that have been inoculated with a mixture of spores of fungi known to colonise paints to humid conditions, free of external nutrients (although these can be added with the inoculum if necessary) for up to 12 weeks (see Figure 4). The resulting growth on

BS3900: Part G6

Method Overview: Replicate sub-samples of both treated and untreated variants of each coating are sprayed with a suspension of spores of a range of fungi known to colonise surface coatings. The samples are then transferred to a humid chamber and incubated for up to 12 weeks. The extent of growth is assessed using a rating scale and this, as well as photographs of the panels, are presented as the results



0 = no growth, 1 = trace to 1% cover, 2 = 1 - 10% cover, 3 = 10 - 30% cover, 4 = 30 - 70% cover and 5 = > 70% cover

There is no pass / fail criterion in the standard but many workers in the coatings industry consider that growth represented by a rating of 2 is the maximum that would normally be tolerated. An example of growth on an untreated coating at a rating level of 5 is shown above.

Figure 4. Example of a Simulated Growth Test

untreated coatings has a visual appearance very similar to that observed in practice and pre-exposure, leaching or artificial weathering can be used to help explore service life. A comparison can be made between treated and untreated variants of a formulation. A similar test, that forms the basis of many of the military standards and specifications, is EN 60068-2-10:2005 / BS 2011-2.1J: 1989 (see Appendix A), and is applicable to a wider range of materials. Again, samples are inoculated and incubated under conditions intended to simulate real life or at least be optimal for fungal growth.

31. Modifications of these methods have been made to allow them to study the effects on algae (the IBRG algal test method for surface coatings) and, less commonly, bacteria.

32. The above methods are indeed very useful and provide valuable information especially for specific material / biocide combinations and can be correlated in some cases to service expectations. However, they can take a long time to perform and, in many cases, need to be adapted in some manner to accommodate a specific material.

Laboratory Tests Based on Artificial Growth Media

33. By far the most commonly used methods for studying the performance of biocides intended to protect materials are those based on artificial growth media such as agar plates. For example, both ISO 846: 1997 and ASTM G21-09 are used widely in the plastics industry to measure the performance of fungicides in formulations (whereas, the more recent, ISO 16869: 2008 is used less widely). ISO 846 allows for studies into the susceptibility of plastic formulations to fungal and bacterial deterioration by attempting to make the plastic the sole source of nutrients (C and N) for the organisms used (even though an agar-based material is employed), as well as providing a variant that provides an external source of nutrients. Although this standard does little to mimic real-life conditions, it can demonstrate whether a material is susceptible to microbiological deterioration or not and whether a biocide can prevent such biodeterioration (provided an untreated variant is included). It also includes a simulation test variant in which samples are buried in soil and then examined for loss of weight and strength (which is extremely useful in industries manufacturing pipes and cables).

34. Although making the plastic the sole source of nutrients might seem like the ideal way to examine the ability of a biocide to protect the material, in many instances it is the presence of soiling that leads to colonisation and subsequent damage to the polymer (sometimes referred to as bio-corrosion). Thus, for certain polymers, the presence of external nutrients is an essential prerequisite in determining the efficacy of a biocide in protecting it. In many instances a consortium of organisms is required to effect colonisation and deterioration of the material and, in general, methods that employ consortia should be selected over those that do not. Similar testing technologies as those used for plastics exist for certain textiles, paper and surface coatings. The most common are listed in Appendix A and a description of the basic principles involved is given in Figure 5 using ASTM G21 as an example.

35. When selecting the appropriate method, consideration must be given to the release mode characteristics of a particular biocide / material combination. Some biocides have a very low solubility in water and hence are emitted at a very low rate from a matrix. This may be sufficient to protect a material that is inherently highly susceptible and which microorganisms may penetrate and colonise. However, if a test (e.g. ISO 16869 - Appendix A) relies on the emission of the biocide from the matrix to measure the effect, it would indicate that such a biocide has no function. Other materials, which are damaged by growth on their surface (especially where soiling is present) due to the production of extracellular enzymes, may fail to be protected by a biocide with such a low emission rate. Thus, the choice of method will be highly dependent on the characteristics of the material as well as the biocide.

Basic Requirements for a Valid Test – Protection

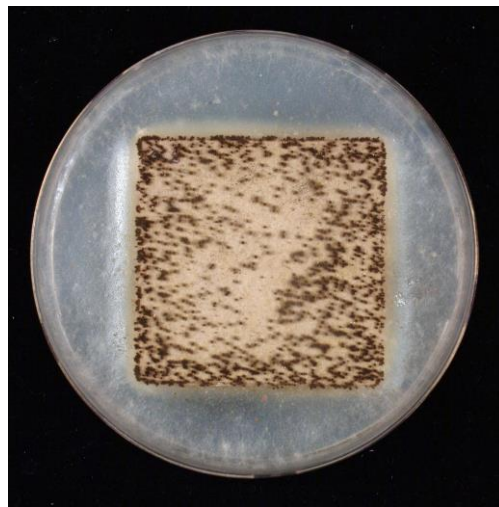
36. Although there is no method that is universally appropriate for all products, even when considering plastics alone, there are some important basic characteristics related to the measurement of activity that need to be taken into consideration. Even if there are many details that vary from case-to-case, there is one basic principle that applies to all: If the purpose of a biocide in a material or article is to prevent changes to it or to extend its durability, then growth

ASTM G21

Method Outline

ASTM G21 - Method Outline

Replicate samples of both treated and untreated material are embedded in a mineral salts-based agar medium. The sample and surrounding agar are then inoculated with the spores of a mixture of fungal species known to colonise plastics. The plates are then placed into chambers in which the humidity is maintained at > 85% RH for up to 28 days. The samples are then inspected for the presence of fungal growth. Typical growth on an untreated material is shown in the plate below.



Growth on Untreated Plastic

Figure 5. An Example of an Agar Plate Based Test

on, or changes to, the untreated material due to the organisms being targeted must be demonstrated in the test.

37. The array of possible material and biocide combinations is vast and phenomena observed in practice cannot always be reproduced in the laboratory, even in a simulation test. A model test matrix would be entirely suitable in many circumstances, provided that the same material is used for both the untreated and treated variants and that it is relevant to the intended use. For example, plasticised PVC and polyurethane would be useful models for rigid or semi-rigid polymers and a room temperature vulcanised silicone would provide a useful model of a sealant etc. Relevance is the key factor. Thus, if a treatment is intended to protect natural fibres in service then a natural fibre should be employed as the model. When more than one type of material (e.g. plastics, paints and synthetic fibres) can be protected by the biocide then representative model test matrices that demonstrate the range of protection anticipated should be considered. This is especially important when their end use will lead to quite different emission scenarios (e.g. in one application the biocide may provide essential protection of a material / article whereas in another it may increase durability while in yet another it may prevent the development of odours in service - see the section on "Prevention of odour", §39 and onwards). The objective is to demonstrate the benefit that a biocide can bring such that a balance against potential risks can be judged. The species employed in any test should be relevant to the intended benefit (i.e. fungi should be employed to demonstrate activity of a fungicide / fungistat etc.). Consortia rather than individual species should be employed (although mixing bacteria with fungi, algae etc. should, in general, be avoided unless it can be justified – e.g. in slime formation). The species employed in the tests should also be relevant to the material under investigation especially where the prevention of the degradation of a material is intended. In many cases the organisms will be specified with the method. Very limited ranges of model / surrogate organisms should be avoided where possible (e.g. the use of *Aspergillus niger* as the sole fungus). The test should include replicates (ideally three or more) for both the treated and untreated variants. In many instances the relevance of the effect demonstrated needs to be explained in some way in terms of the effect required in practice and its duration. This may mean the pre-ageing of samples to provide an example of service life prior to testing.

Basic Requirements for a Valid Test

The following summary provides a guide to the basic requirements for a valid test:

- i An untreated variant of the test material must be included and show the pattern of growth / deterioration that the biocide is intended to prevent at the end of the test.
- ii The test should employ organisms that are relevant to the material / problem being addressed.
- iii Tests that employ a consortium of organisms should be favoured over those that use single species.
- iv A minimum of three replicate test pieces of both treated and untreated materials should be employed.
- v The final data should include either some indication of the impact of service conditions on the performance of the treated material / article or data from an ageing study.

38. A number of worked examples are shown in Table 1 below.

Table 1. Protection – Example Claims, Problems and Testing Approaches

Claim	Example Problem	Example Method
Fungicide is used to treat paper goods to prevent mould growth in service.	Labels used on wine and beer bottles become degraded and stained by fungi and difficult to read when stored in cellars and cool stores.	ASTM D 2020-03 Samples of untreated material should demonstrate a high susceptibility to fungal growth in the test. Treated samples should be free of growth.
Biocide with fungicidal and bactericidal properties is used to protect PVC sheet materials from spoilage and degradation in service	PVC sheet flooring used on solid floors can become colonised by bacteria and fungi on its under surface. This causes staining, cracking and detachment from the substrate.	ISO 846 Parts A and C. Samples of untreated material should support bacterial and fungal growth. Treated material should be free of growth.
Fungicide used to treat cementitious sheet building materials to prevent mould growth.	Calcium silicate wall boards used in ‘dry-wall’ construction can become colonised by fungi. This can occur due to water ingress but is especially problematic shortly after construction when the building is drying. Growth on surfaces in wall voids can give rise to musty odours and the production of fungal spores can cause allergic reactions in sensitive individuals.	BS3900 Part G6. Untreated material should show fungal growth with a rating of 5 after incubation. Treated materials should show growth with a rating no higher than 2.

Prevention of Odour

39. **Overview:** The purpose of this section is to examine claims related to the prevention of odour. This can be seen as being related to the protection of the function of a treated material / article.

40. The control of odour tends to fall into two distinct groups. In the first the odour is caused by growth of microorganisms on a material or article (e.g. surface coatings in bathrooms) or associated with the use of the article (e.g. pipework associated with pools and spas). Usually, odour is a side-effect of microbial growth and will not be produced when microbial growth is inhibited. Specific odour-control claims will usually not be made.

41. In the second group a material (usually a textile) is treated with a biocide in order to inhibit the biotransformation of human (or animal) bodily emissions into odour compounds. The intention is often to extend the wear period for garments (especially those that cannot be washed easily) and to improve the quality of the ‘wearing experience’. Information related to durability (especially the impact of laundering) is vital to making risk – benefit analyses in many cases.

42. The prevention of odour in garments and shoes cannot be modelled easily. Data from wearing trials and the like must be used to demonstrate benefits, although some laboratory-based tests can be used to provide supporting evidence. Tests that can be employed as supporting evidence are listed in Appendix B.

43. The growth of microorganisms on materials results not only in mechanical changes to their properties or alterations to their appearance but can generate unpleasant odours. Aside from being unpleasant to live with, these odours can result in the tainting of food, water and other beverages. They may even render, otherwise serviceable, goods unusable. In many instances, preventing the growth of microorganisms that visually alter the appearance of a material or surface will also eliminate any odours associated with it. For example, the growth of moulds on the surface of paints and sealants in humid locations (e.g. a domestic bathroom) will often lead to the production of musty odours. These, and the release of spores from the growth, can even trigger allergic reactions in sensitive individuals. The addition of a biocide to the paints and sealants can inhibit such growth (or extend the time for which the material remains free of it) and, in so doing, prevent the formation of the odour. In many such examples, the odour, while undesirable, is the consequence of the growth on the substrate and is not the primary target of the treatment but an additional benefit. Such effects are common to many materials that become spoiled by the growth of microorganisms such as tents and awnings.

44. Some spa systems, such as whirlpool baths, can, when used infrequently, start to produce foul odours due to microbial biofilms forming within the pipe-work they contain. Treating these with disinfectants in the same manner as one might treat a drain is usually the recommended solution, but is often short-lived. Attempts have therefore been made to use polymer systems for the pipes etc. that have been treated with biocides to prevent colonisation and thus prevent the generation of odours and deposits. The cause of the odour is the colonisation of the material and the growth that occurs. The odour is the side-effect and, as with the others, the prevention of the growth that causes it can usually be demonstrated using the techniques described in §30-32 and §33-35 above.

45. A series of applications where the production of odour is not necessarily associated with extensive, visible or even measurable microbial growth is in footwear, apparel, certain disposable items (e.g. nappies and incontinence pads) and other, textile, fibre and foam-based items such as towels, bedding, mattresses, mattress covers and carpeting.

46. Although many products make claims related to the prevention of odour, it might be difficult to determine through use (at least within a reasonable time-frame) whether the product delivers the effect promised or not. Thus, a mechanism is required to determine both whether the claims being made can be substantiated and whether the type and amount of treatment used to achieve it is appropriate. Although some instrumental methods have been proposed for measuring various odours, at present they cannot be considered robust and reliable and the only reliable means of determining effects on odour in apparel are those achieved through organoleptic panels and user trials.

47. Attempts have been made to simply associate the measurement of an anti-odour effect with the inhibition of growth of the microorganisms that cause the odour. This slightly indirect approach is probably acceptable in situations where significant growth of organisms is the cause of the odour such as in the prevention of odour in carpets, mattresses and the like. In these cases the service life and the demands on the biocidal treatment can be predicted and modelled. For example, a biocidal treatment may be applied to the backing of a carpet intended to prevent fungal growth resulting from dampness and condensation. The carpet might be anticipated to be wet-cleaned by machine a certain number of times while in service and so a

growth inhibition test similar to those described in sections §30-35 could be used to determine a sensible minimum concentration of biocide to employ.

Control of Odour in Garments

48. By far the largest range of treated materials and articles that claim to prevent / reduce odour are in footwear and apparel, whether worn in intimate contact with the skin or as outer garments. Some of these garments will be subjected to regular laundering (e.g. socks, underwear and sports garments) whereas others will only be cleaned irregularly, if ever (e.g. shoes, ski jackets etc.). Although a plethora of terms may be used to describe the benefit that such treatments bring, ('stays fresh longer' and the like) they are all making a claim related to the prevention of odours, whether foot odour, body odour or mustiness (e.g. resulting from sportswear left in a holdall, towels that remain damp for extended periods of time, maybe after laundering etc.).

49. Some manufactures will have performed wearing trials to assess how well odours are suppressed, possibly in association with various cycles of laundering. However, in the majority of cases the prevention of odour is measured indirectly by measuring microbial inhibition. In the majority of applications the prevention of odour being formed in a garment or shoe is achieved by inhibiting microbial metabolism rather than by killing a population. In many circumstances, simply reducing the rate of biotransformation of compounds present in human sweat is sufficient to achieve the effect desired. There is a significant risk therefore that applying tests intended for protection of materials from degradation will lead to over-treatment. This would lead to an increased exposure of the person wearing it, as well as increased emissions to the environment either during laundering or at the end of its service life.

Testing Methods

50. With most of the biocidal functions considered in the above section "Protection of a material/article and its properties", test conditions simulate in-use conditions rather well and the effects of microbial growth or activity can be observed quite easily. With the control of odour, this is much harder to achieve in a laboratory test, as odour cannot be measured in a simple manner. Thus, an accurate measure of function can often only be achieved through a field trial.

51. Work is in progress to produce tests that simulate specific odour production scenarios in the laboratory. An example is the production of iso-valeric acid, one of the dominant compounds associated with foot odour; tests have attempted to measure the capability of a biocidal treatment to inhibit the bioconversion of L-leucine to iso-valeric acid. Similar tests looking at the bioconversion of urea to ammonia are also under development. However, robust and validated protocols have not been devised to date. Thus, at present, the methods that are employed act as bioassays to measure the presence of a biocidal treatment rather than predict its performance in service. Moreover, because they are bioassays, care must be taken that the test parameters employed do not impose unnecessary demands on the delivery of the biocide. Attempts have been made to correlate the results of such assays with the results of odour trials but this has not proved so far to be a useful general approach, as the results tend to be highly material and biocide related. However, in most cases, as the amount of activity required in practice to inhibit the production of odour is low, these bioassays are useful in measuring the effect of laundering on the loss of activity etc. and thus can at least support the development of products and help ensure that textiles and apparel do not become over-treated.

52. There are two major types of test that have traditionally been used with textiles (and related materials). The first major group employs agar plates in a similar manner to that described in §33-35 and the other major group looks at the impact of a treated textile on populations of (usually) bacteria applied to it as a suspension in an aqueous medium. An overview is given in Appendix B.

53. The agar plate-based tests have almost no useful utility in measuring effects intended to control odour in textiles. Such tests rely on the biocide migrating from the textile into the agar medium at sufficient concentration to inhibit the growth of bacteria either seeded into the agar or placed onto it (see Figure 6). The diffusion characteristics vary hugely from one biocide to another and from one textile to another and the growth medium itself presents a large soiling load to be overcome by the biocide. Larger areas clear of growth are often associated with more potent effects but they could be attributed equally to differences in the leaching rate of a biocide from a material. These types of tests do measure inhibition but on a scale usually significantly larger than required to prevent the metabolism associated with the production of odour. They are therefore not recommended.

54. The second major group, the suspension tests, measure changes in the size of a population following contact with a treated textile. A number of protocols are described in Appendix B. However, most employ relatively high concentrations of nutrients in the suspending medium so that their use, like the agar diffusion methods, can lead to over treatment of textiles. By using lower concentrations of nutrients in the suspending medium and using pre-treatments, such as laundering, these methods can be adapted for use in measuring effects on odour. Such an adaptation has been applied to the methods described in the OECD Guidance Document on the Quantitative Determination of the Antibacterial Activity of Porous, Antibacterial Treated Materials (OECD, 2014) and the IBRG Textile Method (Anon, 2013). These are described schematically in Figure 7 and are based on the ‘germ’ count or absorption phase of ISO 20743: 2007 (Appendix B) where the amount of nutrients present in the cell suspension has been reduced substantially.

AATCC 147

Method Outline: A series of parallel streaks are transferred from an agar plate onto a fresh plate such that they will decrease in density of growth. The test sample or a series of pieces of yarn are then pressed into the agar and the plate is incubated for 24 hours. The effectiveness of the treatment is assessed based on whether it prevents growth where the sample comes into contact with it (and whether a zone free of growth is produced) or not. The inhibition of growth on the different densities of streak are used to assess the potency of the treatment. In this example growth is inhibited from the third weakest streak down.

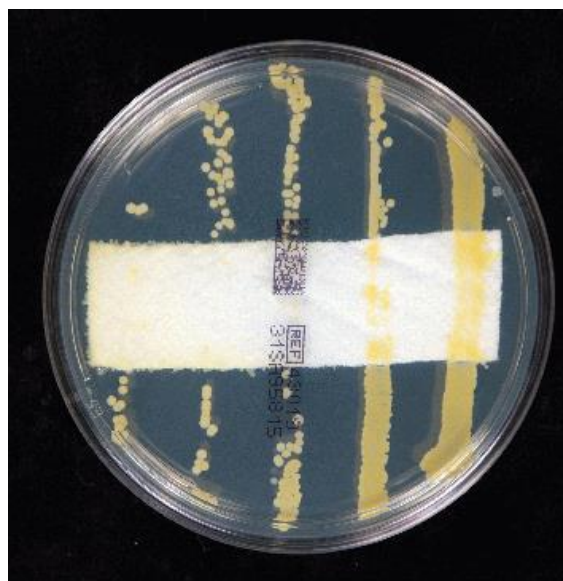
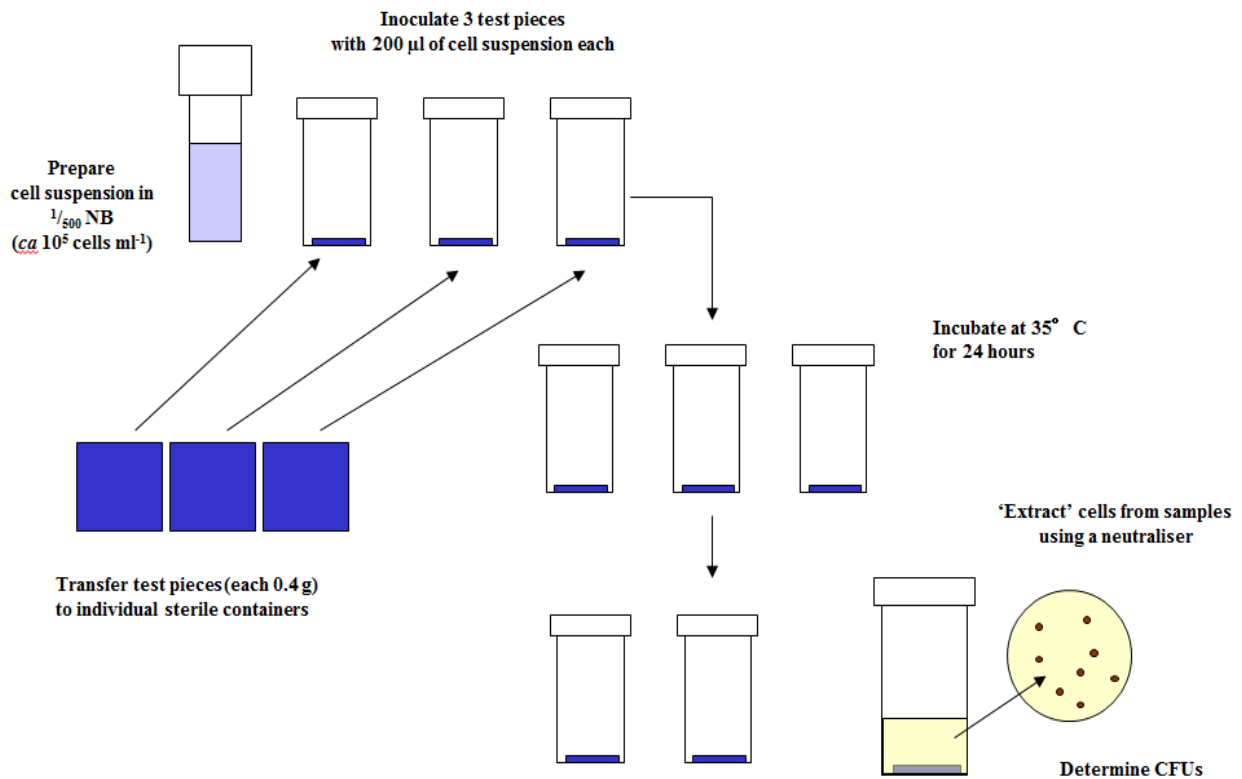


Figure 6. An Agar Plate-Based Test for Textiles

Figure 7. OECD / IBRG Tier 1 Textile Test



55. A biocidal treatment on a textile with an appropriate spectrum of activity (e.g. against a range of Gram Positive and Gram Negative bacterial species such as *Staphylococcus epidermidis*, *Corynebacterium xerosis*, *Proteus vulgaris*, *Escherichia coli*, etc.) would probably inhibit the production of odour. Many such treatments would certainly be capable of producing activity in the suspension tests described above and in Figure 7. But unlike many other biocidal treatments, the problem is not ensuring that the treatment gives sufficient effect but in ensuring that it does not provide too much. Therefore, tests adapted to textile treatments such as the OECD Tier 1 method and the IBRG Textile method (Figure 7) are preferable.

56. In general, the effects required to prevent the formation of odour in shoes and apparel are subtle. It is often sufficient to either inhibit growth or metabolism to prevent the production of odour compounds and a killing effect may not be required. The greatest demand on them is usually in maintaining activity following multiple laundering cycles rather than the demands placed on them in service and the article is not usually intended to deliver a biocide to the skin to prevent odour being formed there. The treatment should be sufficient to achieve its effect for the claimed purpose and not more. Care must be taken to minimise excess exposure of the skin of the wearer and the environment through emissions of the biocide in use and during cleaning and at the time of disposal. The use of over-demanding test protocols, either due to unnecessarily high concentrations of soiling agents and interfering substances or arbitrary and large kill requirements, can lead to over-treatment and excess emissions to the skin and the environment.

57. At present the only truly reliable methods for demonstrating functionality is through replicated and statistically designed wearing trials. The type of bioassay described above can

provide useful data related to durability etc. in support of these but care must be taken when interpreting the data they produce. For example, a treatment may be applied to only certain parts of a garment or shoe or it may be present on only a certain number of filaments in the weave of a textile. In the bioassay, the inoculum is dispersed throughout the whole of the sub-sample of textile and any active substance released would be able to migrate throughout that inoculum whereas in use, this may not occur.

58. In many cases, a large fraction of the active substance incorporated in a textile is lost during laundering, often in the first laundering cycle, either through emission of loosely or only partially bound material or associated with loss of fibres (lint). Thus, the emission rate is rarely continuous either to the environment or to the wearer. It also means that there is potential for active substances to be transferred from treated materials to non-treated materials when laundered together. Again, low demand bioassays (as well as chemical analysis) can be useful in measuring the impact of these emission phenomena and potential cross-transfers. As actual usage would employ a wide range and mix of textile types with a wide variety of anticipated demands and expectations of durability, it would be difficult to address every potential combination and garment type. However, studies on typical textile blends could be used to provide such data to help in the process of assessing risk vs. benefit. Some examples are given in Table 2 below.

Table 2. Odour: Example Claims, Problems and Testing Approaches

Claim	Proof Required	Example Method
Carpet is treated to prevent odours caused by mould growth.	Data should show that the treated carpet does not support fungal growth whereas the untreated one does. The effect should be shown to be sufficiently durable.	A method such as AATCC 174 can be used to demonstrate resistance to fungal growth. For active substances that do not migrate from the fibres / backing a cabinet-based simulation test may be more appropriate. Activity should be shown to persist following simulated ageing.
A sports vest is treated to inhibit the production of odour.	Data from a field trial should show that odour is reduced in treated sports shirts when compared with untreated ones. The effect should be shown to be of sufficient durability during service life to match any claim made.	Wearing trial or scientifically valid odour based simulation study. A comparison of the effectiveness both before and after simulated ageing/washing should be performed. This could be performed either through field trials, simulation tests or the use of a test such as the OECD Tier 1 method. The latter could be used to demonstrate that sufficient activity is still present after washing/ageing to elicit an antimicrobial effect.

Materials and Articles Treated to Add New Properties and Functions not Related to the Primary Use

59. **Overview:** The purpose of this section is to examine claims related to the introduction of new, predominantly hygiene-related, claims. Typical claims associated with these materials and articles include; ‘anti-bacterial’, ‘hygienically clean’, ‘free of bacteria’, ‘prevents the

spread of hazardous bacteria', etc. In some legislations specific requirements are set out for the type of claim which can be made, e.g. Canada's Pest Control Products Act does not allow for claims that are "false, misleading or likely to create an erroneous impression regarding its character, value, quantity, composition, safety or registration". In the US certain claims and phrases that are considered to be related to health either cannot be used or will require the article to be registered and demonstrated to be effective using an agreed protocol.

60. Although a large number of basic efficacy tests exist, none are suitable in supporting such claims; in fact, tailored approaches and adaptations must be employed. In the majority of instances the claim made for the final article will be the one that will require demonstration. This should be taken into consideration when looking at masterbatches that contain a biocide as the final use of these could be highly diverse and examples that cover the range of uses anticipated should be employed (e.g. exposure scenarios that cover wet and dry conditions as well as uses that encompass both preservation and the introduction of added functionality).

61. In the sections "Protection of a material/article and its properties" and "Prevention of odour", the benefit of the use of the biocide is clear and can often be measured in a manner that reflects its purpose, even though in the case of odour control reliance must be made of data generated through in-use trials. However, the case is less obvious for treated articles and materials in which a biocide has been incorporated to add new properties that are not related to the primary function of the article or material in question. This includes such effects as hygienic properties and the like.

Determining the Purpose of the Treatment

62. The very act of including a biocide and introducing antimicrobial properties is often perceived as being desirable, but it is rare that data demonstrating the benefit is presented. The effects envisaged often respond to perceived or hypothetical hazards and problems rather than ones that have been identified and quantified. In some cases performance against a standard may have been shown but the relevance in practice is rarely demonstrated.

63. There are no internationally accepted criteria by which performance can be judged nor, in many cases, are there accepted limits for the number and types of microorganisms that should be present on surfaces and materials in general. It is therefore very difficult to assess likely benefits from such data.

64. Of all the claims made for antimicrobial effects for treated materials and articles, those made for hygienic effects and benefits are probably the most diverse and also, at present, most lacking in the data to support them. Equally diverse is the range of final products that will be manufactured and end uses to which they will be put. Unlike the materials and articles dealt within the sections "Protection of a material/article and its properties" and "Prevention of odour", the effects of articles with a hygienic claim cannot be detected by changes in appearance, mechanical properties or odour. The consequences of their failure are not readily apparent and so it is hard for an end-user / purchaser to determine whether the added value they might expect is being delivered. In fact, in many cases, it is even unclear whether there is actually even an issue that the product is supposed to address (e.g. do bacteria on a mobile telephone present an actual hazard?). There is a need for a clear demonstration of whether the effects that the products claim to deliver are substantiated and justified and why they can be expected to bring a benefit (i.e. what is the problem they are addressing?).

65. To add to this complexity, it is probable that the owner of an active substance will not necessarily be aware of, or be able to anticipate fully, the manner in which it will be employed. In a typical example, an active substance manufacturer will supply a company that will

manufacture a range of plastic masterbatches containing it. These will then be marketed to a diverse selection of manufacturers creating articles with a wide range of end uses, from household goods to medical disposables and even garments and soft furnishings. Such products will have a wide diversity of end applications and be subject to a wide range of environmental conditions and usage patterns. As a result, they will be required to demonstrate a large array of different antimicrobial effects, at least in scale and speed of action, even though the same active substance is present in each. It is often the marketing entity that will provide the support to the final end user and it is these, along with trends and ‘innovations’ within the various industries, which drives the diversity observed. On the whole, it is in this way that the claims observed in the market have become so diverse and so disconnected with the effects that may be achievable. Some of the companies marketing the masterbatches, especially when these are associated with their brand name, apply controls over the claims made in an attempt to ensure that they can be justified. However, such an approach is far from being universal.

66. When someone produces a bedside cabinet for use in hospitals that ‘reduces infections’ by ‘killing bacteria on contact’, one would be expected to provide data that demonstrates that is exactly what the product does. Usually, the antimicrobial active substance applied has undergone a range of laboratory-based, break-point inhibition tests (see Appendix C), and has been shown to be active against organisms such as *Pseudomonas aeruginosa* and Methicillin Resistant *Staphylococcus aureus* (MRSA). However, this does not provide the evidence required. Even if a masterbatch fortified with the active substance has been incorporated into a matrix, which has been tested and shown to demonstrate activity against *Escherichia coli* and *S. aureus* using ISO 22196 (see Figure 8 and Appendix A) this does not mean that the bedside cabinet will fulfil the claims promised. It merely shows that the active substance, when incorporated into a matrix and maintained under wet conditions at 35°C for 24 hours, can be released and affects bacterial growth / survival in the presence of a low level of nutrients. Neither of these sets of data demonstrates how the treated article kills ‘bacteria on contact’ nor how this effect can reduce infections. What is required is data that shows that microorganisms, when deposited by skin contact (even under simulated conditions) and through fine aerosols, are killed within a time-frame that would prevent the surface becoming a vector for cross contamination. This should be combined with studies on the effect of cleaning and disinfection regimes to show that it is sufficiently durable for the end use. It is because of the complex interactions and wide diversity of challenges presented that the use of standardised challenges and required levels of response such as those used in the testing of hard surface disinfectants cannot be employed to describe adequately the effects claimed for treated articles.

Supporting Claims

67. As the combination of end use scenarios and claims for articles treated to introduce new properties is so diverse, efficacy needs to be demonstrated on either the individual article or groups of articles with very similar claimed functionality (or at least models them, in the case of masterbatches). In many cases data on active substance performance and performance of the treated material would need to be supplemented by data specific to the final article, its exposure scenario and the claim being made for it (see Table 3). This would ensure that the conditions of exposure, contact time and target species were appropriate to the end use. It is vital that the benefit that the treated article is intended to deliver be described clearly and the manner in which the efficacy data provided demonstrates this be made clear. The methods used will likely need to be customised to the specific claim but will probably encompass one or more of the groups discussed below. Similarly, durability related to the use of the article will need to be considered as this will often constitute part of the claim.

Effects Intended to either Inhibit Growth or Survival

68. A common claim is that a treated article prevents the growth or survival of microorganisms, especially bacteria, on it. In many cases specific species are quoted (e.g. MRSA) and often these are ones that are associated with diseases in man and livestock (and occasionally plants). To make this a realistic objective it presupposes that the target microorganism will actually either grow or survive on that material or article when it is in service. A paint intended for use in the corridors, wards and operating theatres of a hospital may claim to have been treated to prevent the growth of disease causing bacteria on it, whereas it is more likely to be the absence of sufficient moisture that is the main reason that they are unlikely to grow (McEldowney and Fletcher, 1988). Thus for any claim that a product inhibits growth, it is vital to demonstrate that growth on the material / article in question does occur under its normal conditions of use (not in some artificial laboratory test such as ISO 22196 - Appendix A).

69. Many microorganisms demonstrate poor levels of survival when exposed to environmental surfaces even when those surfaces do not contain a biocide (McEldowney and Fletcher., 1988) and so, as with growth, it will be necessary to have data that demonstrates that the target species (or at least representative surrogates) do indeed survive on the materials and under the conditions in which the articles are intended to be used. Having identified a situation in which microorganisms do grow or survive and present a hazard, data which shows the beneficial effects of the treatment of a material or article needs to be generated.

70. Under the majority of indoor situations, whether in a domestic, industrial or healthcare environment, most microorganisms will not grow on environmental surfaces due to lack of humidity. Many will remain viable though and under some circumstances continue to metabolise. However, where there is sufficient free water, a source of nutrients and an appropriate temperature, growth can occur even if only at a very slow rate. For example, the ducting associated with air handling systems often has areas where condensation accumulates. Microorganisms reaching these areas can colonise the moist surfaces produced, multiply and be re-released into the air in aerosols. In many cases, although the air handling system will be serviced and cleaned, such growth will go unobserved. It is possible to envisage that the ducting could be manufactured from a material in which a biocide has been incorporated or a coating containing one applied. The condensation of moisture on its surface would release the biocide and prevent microbial growth in any liquid deposits formed. In this example, there are a set of conditions that can be defined in which growth could occur. The environmental conditions are known and the constraints on service life can be estimated. There is even a test method that, with appropriate modification, could simulate the application. For example, sub-samples of treated and untreated examples of the material used in the ducting could be tested using a method adapted from ISO 22196 (see Figure 8). It is likely that organic material would be deposited along with any microorganisms and so the nutrient system employed in the standard may be suitable to simulate this. However, the temperature employed in the standard would probably be too high and the contact time may be too short to be relevant. For example, it might prove that using a temperature of 20°C and a contact time of 48 hours or more would be better suited. The use of the cover film would probably make little difference to the relevance of the data but might prove useful depending on the topography of the test pieces. The objective of the test is to show that under conditions similar to those anticipated in practice that a population of bacteria does grow in the simulated condensate and that the presence of the treatment prevents that (it would be useful to supplement the standard test strains with *Pseudomonas aeruginosa* or even *Legionella pneumophila*). It would be possible to simulate service life by, for example, pre-leaching both treated and untreated samples in water and

increasing the levels of both organic and inorganic soiling materials to describe the limitations of the treatment.

Table 3. Cascade Showing the Various Parties Involved in the Supply Chain for Treated Articles

Party	Data Owned	Example of Efficacy Data Provided
Active Substance producer / agent	Toxicity and ecotoxicity data etc. Basic efficacy data on active substance (spectrum of activity etc.) and for typical examples in Product Types in which the active substance will be employed.	Demonstration of basic antibacterial properties delivered when the active substance is incorporated into a model matrix. For example a paint, a plastic, a textile, a ceramic glaze etc. This could be provided by ISO 22196, ISO 20743, OECD Tier 1.
Marketing Entity / Supplier of a Masterbatch, etc.	Provider of scientifically valid data to substantiate major claims in major matrices. Has relationship with Active Substance producer(s) granting letters of access for toxicity and ecotoxicity data etc. This provides a commercial relationship with the parties. It is a positive advantage for them to be able to provide a good supporting package to their customers	Demonstration of ‘real-life’ claims. For example, simulated splash, skin contact, dry contact etc. A range of example materials would be used (a number of common polymers, ceramic glazes, various textiles). Some simple ageing data would be provided.
Treated Article manufacturer	Provider of any specific additional data required to substantiate claim. Has relationship with Marketing Entity or Active Substance producer(s) (if they deal with them directly) granting letters of access for toxicity and ecotoxicity etc. and major part of claim data (if they do not own it themselves) This provides a commercial relationship with the parties.	Demonstration that the activity predicted by the example tests provided by the marketing entity are still expressed in the final article (i.e. manufacturing processes haven’t affected it) - this could be achieved using a simple test, for example as described in ISO 22196. Data related to use conditions and service life expectation would be produced as well as any data on organisms specific to the end use. For example, does the biocide still work when used in a soap dispenser made of injection moulded ABS? A splash test dataset is used that shows a reduction in viability in 1 hour. Additional activity against MRSA and Listeria is added as the main uses are in hospitals and chicken processing factories.

71. Another example where the effectiveness of a treated material might be simulated by an existing method is in the inhibition of growth of *Listeria monocytogenes* in food production environments. Although food production facilities are cleaned and disinfected on a regular basis (in some cases after every shift of 8 hours), it has been noted that persistent reservoirs of organisms such as *L. monocytogenes* remain. It has been hypothesised that such reservoirs are one of the factors related to the sporadic outbreaks of food poisoning caused by this organism

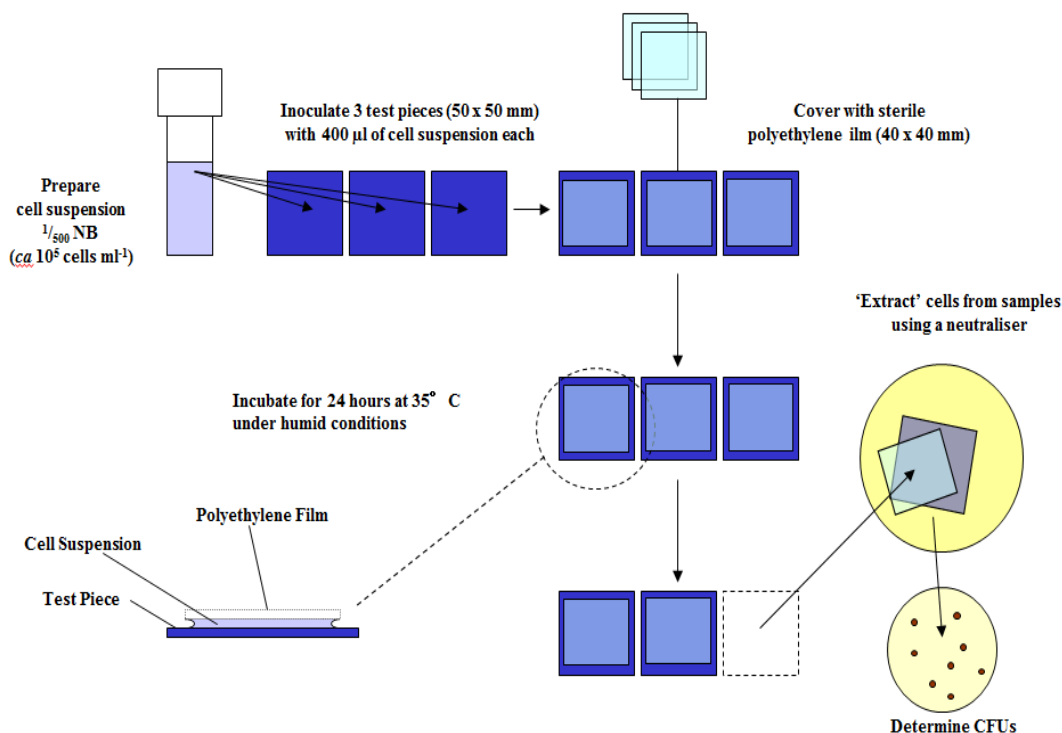
(Holah, Bird and Hall, 2004), especially in cooked and chilled ready meals. It has been considered possible that treated materials may offer a means of eliminating these reservoirs. As with the air handling example given above, it can be envisaged that a modified version of ISO 22196 could be well suited for this purpose as many surfaces remain wet for extended periods after cleaning processes are applied. The standard organisms could be replaced by *L. monocytogenes*, a temperature more suited to the application would be used (e.g. 10 - 15°C) and a contact time of 8 - 12 hours employed. The effectiveness of the treatment at inhibiting the survival and growth of the organism could thereby be modelled. External ageing of the samples prior to testing in a manner that simulates the pattern employed in practice would need to be used to determine whether the treatment was capable of sustaining effectiveness for the anticipated lifetime of the material.

72. Another scenario for which the use of a modified standard test could be employed is a washing-up sponge that has been treated to inhibit the proliferation and subsequent distribution of potentially harmful microorganisms. A protocol based on the OECD Tier 1 method (OECD, 2014) described in Figure 7 could be adapted to simulate the end use. As with the other examples, species, temperature and contact time would need to be changed and information on the impact of ageing on the effect would need to be included, but a model could be devised to describe the functionality of the final article.

73. In the above examples, the effect claimed can be linked to a scientifically valid benefit but this may not always be the case. Simply because a claim can be demonstrated does not mean that a benefit will follow automatically. It is therefore vital that the actual benefit that results from the effect claimed be made clear in the submission.

74. In a related example, *L. monocytogenes* has been shown to grow, albeit slowly, in deposits of food and in condensation on the interior liners of domestic refrigerators (Maktabi, Jamnejad and Faramarzian, 2013). Studies have been presented that this does not occur on treated liners using an approach similar to that described above. However, it is unlikely that such growth will be a significant factor in the transfer of this organism from one foodstuff to another. There will be more significant routes of transfer (e.g. placing cooked foods onto a shelf previously occupied by uncooked poultry meat). These would need to be taken into consideration when assessing risk vs. benefit.

Figure 8. ISO 22196: A Test for Measuring Basic Antibacterial Properties of Non-Porous Materials



75. Clearly there are methods that can be employed to explore the functionality of materials and articles that have been treated to prevent the growth of organisms that pose potential hazards to man and livestock. However, the key to understanding the benefit they may bring, is to be able to show that growth will occur under the normal conditions of use (and possibly storage – e.g. growth of bacteria on the bristles of a toothbrush between uses) and that the treatment can prevent this under those same conditions within a time frame that is relevant to the application. The benefit of the treatment needs to be described clearly such that a valid analysis of risk vs. benefit can be made.

ISO 22196

Method Outline: An aliquot (usually 400 µl) of a log phase cell suspension of either *Escherichia coli* (ca10⁵cells/ml; ATCC 8739) or *Staphylococcus aureus* (ca10⁵cells/ml; ATCC 6538p) in 1/500 Nutrient Broth are held in intimate contact with each of 3 replicates of both treated and untreated variants of the test materials using a 40 x 40 mm polyethylene film (e.g. cut from a sterile Stomacher bag) for 24 hours at 35°C. The populations are then recovered using a neutraliser solution validated for the active substance(s) present and the size of the surviving populations are determined as colony forming units (CFUs) using a dilution plate count method. Additional replicate unfortified samples are also inoculated in the same manner but are analysed immediately to determine the size of microbial population present prior to incubation. The differences between the initial and final population as well as from the treated and untreated materials are used to assess the basic antibacterial properties of the test materials.

Applications that Intend to Kill Organisms through Contact

76. One of the major aspirations for treated materials and articles that introduce new properties is to kill supposedly harmful organisms. In many instances it is not entirely clear what hazard such organisms might present and, in many cases, evidence that they are present is not even available. Therefore the potential hazard that is being addressed must be defined.

77. Microorganisms will come into contact with environmental surfaces, textiles and the like through both contact with other materials, foodstuffs, soil and via contact with the skin. They will also be deposited from the air (either by simple, dry settlement or through the deposition of aerosols) as well as through spillages and splashes of liquids etc. or even combinations of these routes. The size and composition of the populations deposited will vary enormously from a few cells per m² of a single species to hundreds of millions per cm² or more of a wide range of species, depending on the circumstances. In some instances the deposition will be associated with other organic and / or inorganic material, whereas in others little additional material will be present. Thus, the potential demand on the function of treated materials is highly varied both in type and scale. This variation will have a profound impact on the ability of a treatment to produce an effect. For example, if a treatment delivers its effect by being emitted from the surface, as will be the case for the majority of active substances, then the emission needs to be triggered somehow. There must be a way for the active substance to interact with the target organism that has come into contact with the material / article. In the majority of cases, water is the crucial component to facilitate such release and transfer. If the event that caused the deposition does not introduce moisture and the normal exposure conditions of the material or article are dry (or only subject to normal, ambient indoor humidity), the ability of a treatment to produce an effect will probably be limited.

78. The benefit envisaged for treated materials and articles that claim to kill are diverse. They encompass surfaces that are intended to slowly reduce a population over time to those that propose to prevent cross contamination via surfaces that are touched regularly and thus must deliver their effect quickly (e.g. door handles).

79. As with all of the applications for treated materials and treated articles, the first step to being able to show their benefit is to define the problem that they address. When attempting to eliminate or reduce populations coming into contact with or present on surfaces one needs to determine the hazard that their presence implies. For example, are the background populations found on environmental surfaces a cause for concern? In a high dependency ward in a hospital, the presence of bacteria on surfaces that are touched frequently may be of concern but these are less likely to be of consequence in an office or domestic environment. Similarly, the floors and walls will likely be less critical as vectors than the touch-screen of a heart monitor. Does the inclusion of antimicrobial properties into an article bring useful functionality to the article or are there other/better practices in place (e.g. regular disinfection of surfaces) to address the hazard?

80. For example, if a patient in a hospital has severe diarrhoea or vomits over a surface or into bedding, the medical staff will intervene. They will clean the patient, replace bedding and bedclothes etc. and clean and disinfect the areas affected. However, either during the event or during the cleaning-up, it is possible that small deposits, possibly quite remote from the main event (or on the uniforms of the staff), may go unobserved and these could contain large numbers of potentially infective organisms. The presence of a treatment in a material (the release of which might be triggered by the arrival of a deposit of liquid) could help reduce or eliminate these, such that the risk of cross contamination from that deposit is reduced. The effectiveness of this would depend on the rate at which such an effect could be realised (given that the deposit will likely dry fairly quickly) and its ability to function in the presence of additional organic material.

81. Cross-contamination through surfaces that are touched with a high frequency such as door handles present similar issues. The size of the populations deposited by any one individual will likely be low and it will probably be associated with only a small amount of moisture (this may be higher in some situations such as at the exits of washrooms). In addition, the interval between one person touching the handle and the next could be very short. Thus at least two variables (contact time and moisture) must be taken into consideration when designing a protocol intended to demonstrate the effectiveness of the treatment.

Figure 9. Example Test Protocol for Measuring the Activity of Non-Porous Treated Materials on Splashes of Contaminated Liquids

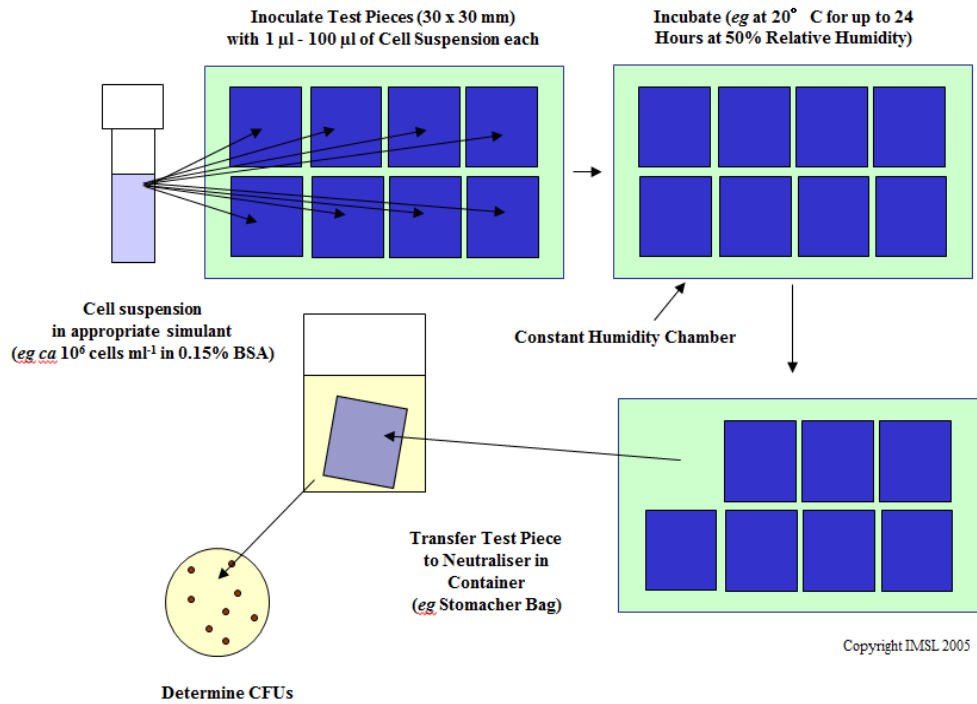
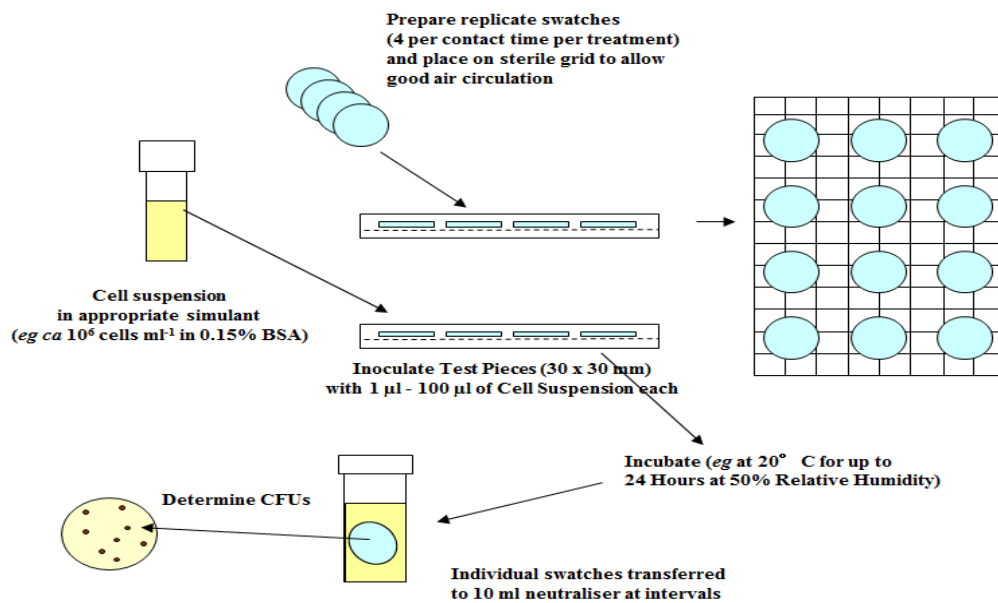


Figure 10. Example Test Protocol for Measuring the Activity of Porous Treated Materials on Splashes of Contaminated Liquids



82. Although the standards that measure the basic antibacterial and antifungal (even antiviral) properties of treated materials can be modified to simulate applications that intend to inhibit growth, none of them provide a methodology that can be adapted for end uses related to the killing ability of non-porous materials. However, ISO 20743 (Appendix B) contains methods that explore the impact of treated textiles on simulated dry contact and these can be adapted for some claimed effects on non-porous materials. There are a number of approaches that have been described in the literature (OECD, 2007) and some of these are undergoing further study by the International Biodeterioration Research Group³ and have been described, in principle at least, by the OECD Working Group on Biocides⁴.

83. The approach that needs to be taken to validate claims that a treated material or article kills a target population requires that the exposure scenario be understood and that the benefit that is intended is clear and valid.

84. Of the major scenarios that lead to the contamination / cross-contamination of inanimate surfaces (and, in some cases, textiles and other porous materials) the deposition of microorganisms through the deposition of aerosols and splashes of contaminated liquids and contact by hand are the most significant. These will therefore be considered in more detail in this section and example protocols that address them are given.

Deposition by Aerosols and Splashes

85. Using the example of contamination by splashes, it is possible to design a test protocol which simulates such an event (similar ones can be designed for looking at fine sprays and aerosols). In such protocols (e.g., see Figures 9 and 10), small aliquots of a suspension of bacterial cells (probably in combination with a soiling agent relevant to the end use – e.g. 30 g/litre bovine serum albumin) are applied to replicate treated and untreated variants of the material in question (Figure 9 shows the approach used for non-porous materials and Figure 10 shows the approach used for absorbent materials). The samples are then incubated under conditions that replicate the end use of the article and sub-samples are analysed at intervals to determine the number of viable organisms present on them. In this way the method looks at the impact of the treatment on viability over time and the scale and speed of this can be compared with the demands presented by the end use. The size (and number) of the aliquots employed along with the temperature, relative humidity and contact time can be adapted to simulate a specific range of events. The combination of these would need to be selected such that they match the nature of the exposure scenario and the claim being made (e.g. shorter contact intervals for frequently touched surfaces etc). In this way, a description of the benefit can be produced. A more detailed description of such a protocol is given below for a specific example.

Example Protocol for Simulated Splashes

86. In this example, the antibacterial activity of dry paint films will be determined using a method that simulates the deposition of bacteria in small splashes of liquids. The paint is intended for use in clinical environments and activity against *Escherichia coli*, *Pseudomonas*

³ <http://www.ibrg.org>

⁴ <http://www.oecd.org/chemicalsafety/pesticides-biocides/biocides.htm>

aeruginosa, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Enterococcus hirae* and MRSA are desired. Single splashes of 50 µl will be employed, and exposure conditions of 20°C and a relative humidity of 50% will be used. Contact times of 0, 6, 12 and 24 hours will be employed to study the effect of the treated material on the survival of the test species. One of the key components of the claim being investigated is that bacteria die faster on the treated paint than on a conventional paint. A time-course experiment is therefore required. The basic outline is described schematically in Figure 9.

Test Bacteria

87. Strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Enterococcus hirae* and Methicillin Resistant *Staphylococcus aureus* (MRSA) will be employed in this example. However, other species can be used providing they are relevant to the claim being made. In some instances the additional species will require different methods to cultivate, expose and recover them that are not described in this protocol; however, the conditions for a valid test (see below) apply to all species of microorganism used. Any additional steps must be fully documented in the final report. Any deviations have to be justified and it must be demonstrated that they do not alter the fundamental performance of the test materials in any significant manner.

88. For the standard test species, stock cultures are stored at 5°C ± 3°C on Trypticase Soya Agar (TSA) and transferred monthly. After five transfers or if more than one month has passed between transfers, the stock culture is discarded and replaced with a fresh culture, obtained from either the institute or culture collection concerned or from frozen or lyophilised long-term stocks held by the testing institute.

89. Two days prior to testing, bacteria are transferred from the stock cultures using a sterile inoculating loop to an agar plate of TSA and incubated at 35°C ± 1°C for 16 hours to 24 hours. From this culture, bacteria are transferred onto a fresh agar plate of TSA and incubated at 35°C ± 1°C for 16 hours to 24 hours. This plate will be used to prepare the inoculum used in the test. When conducting testing on numerous lots of materials, multiple plates of an organism can be prepared.

Preparation of Test Materials (including untreated controls)

90. Testing is performed on at least three replicate sub-samples from each treated test material per contact time per species (i.e. a total of 72 sub-samples will be required for this example). The same number of untreated sub-samples (controls) is required.

91. The use of more than three replicates per test species of the treated test material may help reduce variability, especially for materials that show smaller antibacterial effects. When testing a series of antibacterial treatments for a single material, each antibacterial treatment may be compared to a single set of untreated materials if all the tests are conducted at the same time using the same test inoculum.

92. In this example protocol the test coatings are prepared on polyester scrub resistance panels by block spreader to give a film thickness equivalent to that recommended for the product. The films are then allowed to dry for 7 days at ambient temperature and humidity to ensure no volatile components remain in the film. The replicate sub-samples are cut from these coated panels. Each sub-sample measures 20 ± 2 mm × 20 ± 2 mm (the size of the sub-samples employed can be adapted to the volume of inoculum applied).

93. In general, when preparing the samples, care should be taken to avoid contamination with microorganisms or extraneous organic debris. Similarly, the test samples should not be allowed to come into contact with each other. If metal foil is used to avoid cross-contamination

between samples during transport and storage, it is necessary to ensure that the metal does not have any antibacterial effect. If necessary, test samples can be cleaned / disinfected / sterilised prior to testing (e.g. by wiping with a solution of 70% v/v ethanol in water). It should be noted that the cleaning of test samples can cause changes such as softening, dissolution of the surface coating or elution of components and so should be avoided. If cleaning is required due to cross contamination, the cleaning method must be stated in the final report.

94. All sub-samples are placed into a chamber in which the humidity can be maintained at 50% Relative Humidity (RH; or the humidity to be employed in the test) and pre-equilibrated for 24 hours at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for up to 24 hours ± 15 minutes prior to being inoculated.

Preparation of the Test Inoculum

95. Bacteria are transferred using a sterile inoculating loop from the pre-incubated plates described above into a small amount of sterilized distilled water (SDW; other suspending media may be used depending on the claim being made). It is important to ensure that the test bacteria are evenly dispersed and sterile glass beads plus a vortex mixer can be used to achieve this if necessary. The number of bacteria in the resulting suspension is then estimated using direct microscopic observation and a counting chamber or another appropriate method (e.g. spectrophotometrically). One suspension is prepared for each test species. These suspensions are then diluted with SDW, as appropriate for the estimated bacterial concentration, to obtain a bacterial concentration that is between 1.5×10^6 cells/ml and 5.0×10^6 cells/ml, with a target concentration of 3×10^6 cells/ml. These suspensions are used as the test inoculum. The test inoculum is used within 2 hours of preparation and held at room temperature. It should be mixed thoroughly by hand immediately prior to use. The number of colony forming units in the individual inocula is verified by the same dilution plate count method as used for all other stages of the test.

Inoculation of Test Materials (including controls)

96. An aliquot (0.05 ml) of the appropriate test inoculum as prepared in above is transferred onto the surface of each of the sub-samples using a sterile pipette in the incubation chamber (this constitutes the simulated splash). Three replicate sub-samples of each treated and untreated system and species are then removed from the chamber to be used for the 0 hour time point analysis using the method described below and placed into individual sterile containers containing an aliquot (10 ml) of a neutraliser that has been validated for the active substance(s) contained in the treated material. The chamber is then closed and transferred to an incubator.

Incubation

97. The samples in the humidity controlled chamber are incubated at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for up to 24 hours ± 15 minutes.

Recovery of Bacteria from the Test Samples

98. At each contact interval, three replicate sub-samples of each treated and untreated system and species are removed from the chamber and placed into individual sterile containers containing an aliquot (10 ml) of a neutraliser that has been validated for the active substance(s) employed in the treated material. The contents of the containers are then mixed vigorously by hand to ensure that the bacteria have been released from the test samples. Sterile glass beads and a vortex mixer may be employed if necessary.

Measurement of Colony Forming Units

99. The number of colony forming units present in the suspension produced above is determined by dilution plate count onto TSA. Any validated method (e.g. pour plate, spread

plate and spiral dilution) may be employed. The suspensions are diluted in the neutraliser employed such that accurate counts of colonies can be performed. The undiluted neutraliser solution obtained from the treated samples will also be analysed by the pour plate technique using TSA. This must be performed irrespective of the method used to analyse the suspensions from the untreated samples and the diluted suspensions from the treated samples. This is to increase the limit of detection of the method (a membrane filtration method could also be performed if a lower limit of detection was considered necessary for the material under test).

100. For the pour plate technique, transfer an aliquot (1 ml) of each undiluted neutraliser suspension into individual sterile Petri dishes. Molten (and then held at approximately $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$) TSA (approximately 15 ml per dish) is then placed into each Petri dish and swirled gently to disperse the bacteria uniformly. Once set the Petri dishes are incubated at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 to 48 hours depending on the species. The same conditions shall be employed for the plates derived from the other dilutions / method.

101. After incubation the number of colonies present on the plates are counted and recorded.

Results

Presentation of Results and Interpretation of Data

102. The results are expressed as colony forming units per cm^2 (based on the volume and spread of the inoculum employed) quoted as the geometric mean of the data. Microbiological effects should be interpreted based on the difference between populations exposed to the treated and untreated materials. The size of the populations prior to exposure / incubation and after may also be of importance. If differences need to be calculated they should be expressed as the true difference and not as percentage differences. In this example the rate of change is of interest and this can be calculated for the various time points employed. Any differences between treated and untreated samples shall be tested for statistical significance ($p < 0.05$) using a recognised technique. The data will almost certainly require transformation (probably base-10 logarithm) before analysis by parametric statistical techniques such as the t-test or Analysis of Variance (ANOVA) in order to satisfy the Gaussian distribution requirement of these tests. Non-parametric methods can also be used (e.g. Mann-Whitney). The relative power and power-efficiency of the various tests is outside the scope of this protocol and is discussed in standard texts. With the distributions found in biological testing there is often only small differences between parametric and non-parametric methods.

103. The test report must include the following information:

- active substances and their corresponding concentrations in the material(s) tested;
- suspension media and any interfering substances and their concentrations used during the test;
- the details of the materials tested;
- the size, shape and thickness of the test materials;
- the species of bacteria used and their strain numbers;
- the volume of test inoculum used;
- the number of viable bacteria in the test inoculum (CFU/ml);
- the details of the neutraliser employed and the data validating it;
- the results as CFU/cm² data describing the variance of the data and the statistical significance of any differences detected compared to survival on controls / untreated samples;
- a graphical presentation of the data;

- full details of the procedure including temperature and humidity employed, contact intervals, any cleaning/sterilisation of the test samples. The basis of the conditions employed should be supported by relevant scientific explanations and supporting data;
- identification of the test laboratory, and the name and signature of either the head of the laboratory or the study director;
- the date of commencement of the experiments; the date of the test report.

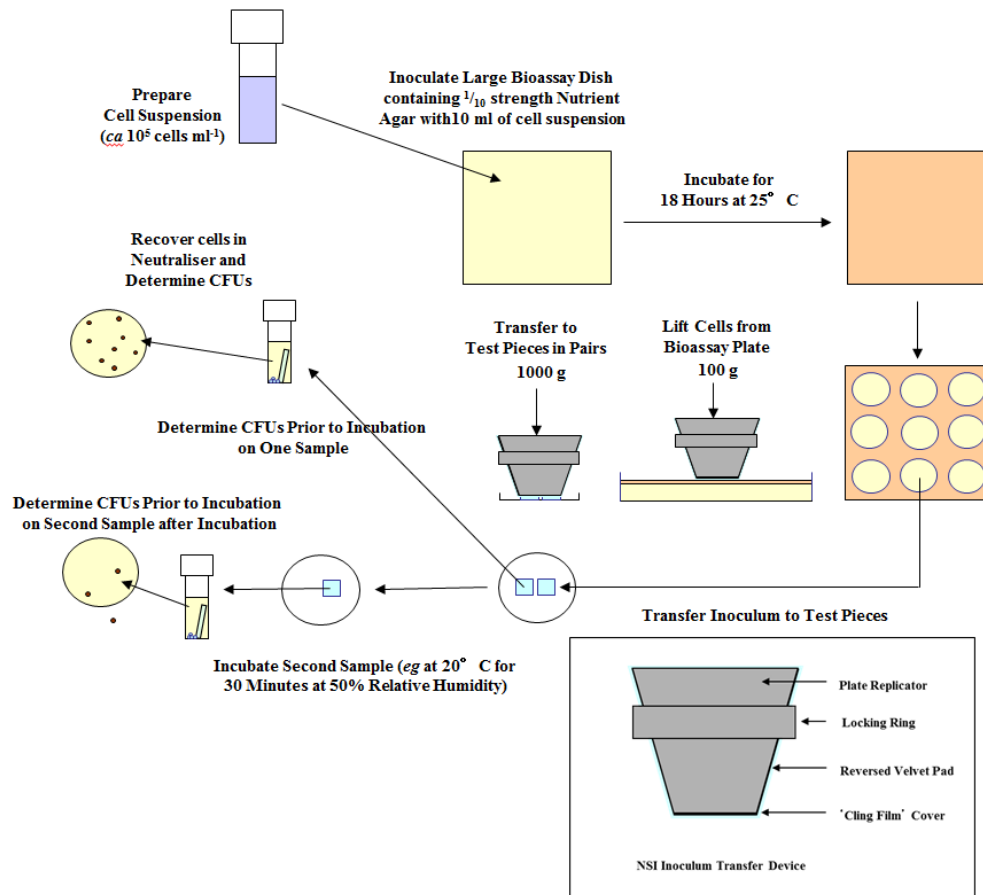
Deposition by Hand Contact

104. The second major route of contamination of inanimate surfaces (and textiles) is through hand contact. This route differs from the deposition by splashes as the amount of moisture involved is usually significantly lower and the frequency of contact between the hand (and different individuals) and the surface can be much higher. As mentioned above, ISO 20743 (Appendix B) contains methods that are designed to limit the amount of moisture involved in the transfer of microorganisms onto a textile surface to simulate either skin-to-textile or textile-to-textile contact. Similar approaches can be described that model the transfer of bacteria from skin to non-porous materials (see Figure 11). By altering contact intervals, test species etc. it is possible in this way to examine the effectiveness of treatments on touch surfaces such as door handles and instrument consoles, keyboards and the like.

Example Protocol for Hand Contact

105. In this example, the antibacterial activity of flexible PVC intended for use as the cover material for the membrane switch interface of monitors used in a healthcare setting will be determined using a method that simulates the deposition of bacteria by hand contact. Activity against MRSA is desired. Bacteria will be transferred to the surface of the test pieces using a non-suspended inoculum and exposure conditions of 20°C and a relative humidity of 50% will be used. Contact times of 0, 15, 30 and 60 minutes will be employed to study the effect of the treated material on the survival of the test species. One of the key components of the claim being investigated is that bacteria die on contact with the surface, and at a rate that is sufficiently fast so that transfer between operators via the surface is minimised. The basic outline is described schematically in Figure 11.

Figure 11. Determining the Antibacterial Activity of Surfaces using a Non-Suspended Inoculum



Test Bacterium

106. A strain of Methicillin Resistant *Staphylococcus aureus* (MRSA) will be employed in this example. However, other species can be used providing they are relevant to the claim being made. In some instances the additional species will require different methods to cultivate, expose and recover them that are not described in this protocol; however, the conditions for a valid test (see below) apply to all species of microorganism used. Any additional steps must be fully documented in the final report. Any deviations have to be justified and it must be demonstrated that they do not alter the fundamental performance of the test materials in any significant manner.

107. For the standard test species, stock cultures are stored at $5^\circ \text{C} \pm 3^\circ \text{C}$ on Trypticase Soya Agar (TSA) and transferred monthly. After five transfers or if more than one month has passed between transfers, the stock culture is discarded and replaced with a fresh culture, obtained from either the institute or culture collection concerned or from frozen or lyophilised long-term stocks held by the testing institute.

108. Two days prior to testing, bacteria are transferred from the stock cultures using a sterile inoculating loop to an agar plate of TSA and incubated at $35^\circ \text{C} \pm 1^\circ \text{C}$ for 16 hours to 24 hours. From this culture, bacteria are transferred onto a fresh agar plate of TSA and incubated at $35^\circ \text{C} \pm 1^\circ \text{C}$ for 16 hours to 24 hours. This plate will be used to prepare the inoculum used in the test.

When conducting testing on numerous lots of materials, multiple plates of an organism can be prepared.

Preparation of Test Materials

109. Testing is performed on at least six replicate sub-samples from each treated test material per contact time (i.e. a total of 18 sub-samples will be required for this example). The same number of untreated sub-samples (controls) is required. The 0 hour contact data is produced for each time interval set at the point of inoculation and so requires no additional samples.

110. The use of more than three replicates per test species of the treated test material may help reduce variability, especially for materials that show smaller antibacterial effects. When testing a series of antibacterial treatments for a single material, each antibacterial treatment may be compared to a single set of untreated materials if all the tests are conducted at the same time. Half of the treated and untreated test specimens are used to determine the number of colony forming units that have been transferred to the test surfaces immediately after inoculation and half are used to determine the number present after each contact interval.

111. Each test specimen shall be 20 ± 2 mm x 20 ± 2 mm and must be flat. Specimens should be no more than 10 mm in thickness. Although it is preferable to prepare test specimens from the final product itself, the topography of the final article may interfere in the efficient deposition of bacteria by the inoculation method employed. In the example given here, the final article is a flat control panel and is less than 1 mm in thickness.

112. In general, when preparing the samples, care should be taken to avoid contamination with microorganisms or extraneous organic debris. Similarly, the test samples should not be allowed to come into contact with each other. If metal foil is used to avoid cross-contamination between samples during transport and storage, it is necessary to ensure that the metal does not have any antibacterial effect. If necessary, test samples can be cleaned / disinfected / sterilised prior to testing (e.g. by wiping with a solution of 70% v/v ethanol in water). It should be noted that the cleaning of test samples can cause changes such as softening, dissolution of the surface of the material or elution of components and so should be avoided. If cleaning is required due to cross contamination, the cleaning method must be stated in the final report.

Preparation of test inoculum

113. Several individual colonies are transferred using a sterile inoculating loop from the pre-incubated plates described above into a small amount of sterilized distilled water (SDW). It is important to ensure that the test bacteria are evenly dispersed and sterile glass beads plus a vortex mixer can be used to achieve this if necessary. The number of bacteria in the resulting suspension is then estimated using direct microscopic observation and a counting chamber or another appropriate method (e.g. spectrophotometrically). One suspension is prepared for each test species. These adjusted suspensions are then diluted with SDW, as appropriate for the estimated bacterial concentration, to obtain a bacterial concentration that is between 1.5×10^6 cells/ml and 5.0×10^6 cells/ml, with a target concentration of 3×10^6 cells/ml. These suspensions are used to inoculate the plates from which the final inoculum will be transferred. The test inoculum is used within 2 hours of preparation and held at room temperature. It should be mixed thoroughly by hand immediately prior to use using a vortex mixer. The number of colony forming units in the individual inocula is verified by the same dilution plate count method as used for all other stages of the test.

114. An aliquot (5 ml) of this inoculum is spread over the surface of a bioassay plate (20 x 20 cm) filled with sterile, molten and tempered (at 45°C – 50°C) 1/10 Trypticase Soya broth

fortified with agar (15 g / litre) prior to sterilisation⁵ and then incubated for 18 hours at 25°C + 1°C (this may need to be adjusted depending on the microorganisms being employed – the objective is to achieve a very thin lawn of growth on the agar). Multiple plates may be required depending on the number of samples and controls to be tested.

Inoculation and Incubation of the Test Specimens

115. A pair of sub-samples is placed into a sterile Petri dish with the test surface uppermost. Bacteria are transferred from the surface of the bioassay plate described above onto the surface of the test specimens using a modified replica plating device with a contact diameter of 50 mm. The standard 'velvet' contact face is covered with a layer of thin, flexible, plastic food-wrap film. A force of approximately 5 g / cm² (achieved by making the weight of the replica plating device 100 g using external weights) is employed to pick up the inoculum from the bioassay plates and a force of 125 g / cm² (achieved by either making the weight of the replica plating device 1000 g using external weights or by applying a downward force by hand and measuring it by placing the Petri dish / sample of a balance) is used to transfer it to the pair of test specimens. A contact interval of 5 seconds is used for both pickup and transfer. A fresh piece of film is used for each pair of specimens.

116. Immediately after inoculation, one of each pair of sub-samples is placed into a sterile container containing an aliquot (10 ml) of a neutraliser that has been validated for the active substance(s) employed in the treated material. The contents of the container are then mixed vigorously to ensure that the bacteria have been released from the test samples. Sterile glass beads (2 mm - 5 mm in diameter) and a vortex mixer shall be employed. The remaining specimen, in its Petri dish, is transferred to a chamber in which the relative humidity can be maintained at 50%. This is repeated for each pair of test specimens and when all of them have been introduced into the chamber it is incubated for up to 60 minutes. The samples must be inoculated as a timed sequence to ensure that the contact time is equal for each test piece.

Recovery of Bacteria from the Test Samples

117. At each contact interval, three replicate sub-samples of each treated and untreated system are removed from the chamber and placed into individual sterile containers containing an aliquot (10 ml) of a neutraliser that has been validated for the active substance(s) employed in the treated material. The contents of the containers are then mixed vigorously to ensure that the bacteria have been released from the test samples. Sterile glass beads (2 mm - 5 mm in diameter) and a vortex mixer shall be employed.

Measurement of Colony Forming Units

118. The number of colony forming units present in the suspensions produced above is determined by dilution plate count onto TSA. Any validated method (e.g. pour plate, spread plate and spiral dilution) may be employed. The suspensions are diluted in the neutraliser employed such that accurate counts of colonies can be performed. The undiluted neutraliser solution obtained from the treated samples will also be analysed by the pour plate technique using TSA. This must be performed irrespective of the method used to analyse the suspensions from the untreated samples and the diluted suspensions from the treated samples. This is to increase the limit of detection of the method (a membrane filtration method could also be performed if a lower limit of detection was considered necessary for the material under test).

⁵ Recipe: Enzymatic Digest of Casein 1.7 g/L, Enzymatic Digest of Soybean Meal 0.3 g/L, Sodium Chloride 0.5 g/L, Dipotassium Phosphate 0.25 g/L, D-Glucose 0.25 g/L, Agar 15 g/L with a final pH of 7.3 ± 0.2 at 25°C

119. For the pour plate technique, transfer an aliquot (1 ml) of each undiluted neutraliser suspension into individual sterile Petri dishes. Molten (and then held at approximately $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$) TSA (approximately 15 ml per dish) is then placed into each Petri dish and swirled gently to disperse the bacteria uniformly. Once set the Petri dishes are incubated at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 to 48 hours depending on the species. The same conditions shall be employed for the plates derived from the other dilutions / method.

120. After incubation the number of colonies present on the plates are counted and recorded.

Results

Presentation of Results and Interpretation of Data

121. The results are expressed as colony forming units per cm^2 (each test specimen has an area of 4 cm^2) quoted as the geometric mean of the data. Microbiological effects should be interpreted based on the difference between populations exposed to the treated and untreated materials taking into account the size of the populations present on each of the pair of samples that was analysed prior to exposure / incubation (these also provide the zero time data). If differences need to be calculated they should be expressed as the true difference and not as percentage differences. Any differences between treated and untreated samples shall be tested for statistical significance ($p < 0.05$) using a recognised technique. The data will almost certainly require transformation (probably base-10 logarithm) before analysis by parametric statistical techniques such as the t-test or ANOVA in order to satisfy the Gaussian distribution requirement of these tests. Non-parametric methods can also be used (e.g. Mann-Whitney). The relative power and power-efficiency of the various tests is outside the scope of this protocol and is discussed in standard texts. With the distributions found in this biological testing there is often only small differences between parametric and non-parametric methods.

122. The test report must include the following information:

- active substances and their corresponding concentrations in the material(s) tested;
- the details of the materials tested;
- the size, shape and thickness of the test materials;
- the species of bacteria used and their strain numbers;
- the number of viable bacteria in the test inoculum used to prepare the bioassay plates (CFU/ml);
- the details of the neutraliser employed and the data validating it;
- the results as CFU/ cm^2 data describing the variance of the data and the statistical significance of any differences detected compared to survival on controls / untreated materials;
- a graphical presentation of the data;
- full details of the procedure including temperature and humidity employed, contact intervals, any cleaning/sterilisation of the test samples. The basis of the conditions employed should be supported by relevant scientific explanations and supporting data;
- identification of the test laboratory, and the name and signature of either the head of the laboratory or the study director;
- the date of commencement of the experiments and the date of the test report.

Adaptability of Methods and Field Trials

123. Using methods like those described above and other, scientifically designed, approaches it is possible to simulate in the laboratory the potential effectiveness of treated materials and articles intended to demonstrate killing effects under the normal conditions they will be used in. The detailed examples provided employ vegetative bacteria but, for the

simulated splash test, the method can accommodate suspensions of viruses, bacterial endospores, fungal spores and even the vegetative cells and cysts of protozoa. Similarly, the hand contact protocol could also be modified to utilise these types of microorganism, although the agar plates used to create the lawn of weak growth of bacteria would be used simply to act as a transfer surface for a suspension of the cells / particles. However, an adapted version could accommodate the growth of yeasts and so be used for these. Additionally, field tests could be performed to determine if, for example, treated surfaces that are touched frequently do have fewer microorganisms on them than untreated ones. Such trials would require large numbers of replicates and fresh examples of both treated and untreated articles that enter service at the same time. Careful consideration would also need to be given to how the effect is measured. For example, if moist swabs were to be employed it is important that the process of sampling does not affect (promote) the action of the treatment. A neutraliser for the active substance(s) that the article contained would need to be employed to prevent this. Having generated data by either a series of relevant simulations and / or field studies, an assessment must then be made as to whether any effects that were observed are sufficiently substantial and of sufficient speed to bring a benefit. Some element of durability testing will almost certainly be required to demonstrate that the benefit is sustained for the intended lifetime of the article.

Basic Requirements for a Valid Test

124. As with all treated material and articles, the objective is to demonstrate the benefit that the treatment can bring such that a balance against potential risks can be judged. Efficacy for articles that have been treated to introduce new properties must be performed on the final product. The application area, materials employed, conditions of use and the benefit intended must be simulated realistically by the testing procedures employed. The results must demonstrate that the effect is sufficient to deliver the benefit intended in terms of speed and scale and organisms relevant to the end use must be employed. If specific organisms are the target of the article they must be included. In general, individual species should be employed to describe effects. Tests must demonstrate the durability of the effect for the proposed lifetime that is claimed. If this is shorter than the lifetime of the article, this should be explained. A number of examples are shown in Table 4 below.

Basic Requirements for a Valid Test

The following summary provides a guide to the basic requirements for a valid test:

- i. The test should be carried out on the final article.
- ii. An untreated variant of the test material must be included such that the impact of the treatment can be demonstrated.
- iii. Test conditions should reflect normal conditions of use in terms of humidity, temperature, contact frequency, durability etc.
- iv. The test should employ organisms that are relevant to the end use of the article and the benefit being claimed.
- v. Tests that employ a single species of organisms should be favoured over those that use consortia.
- vi. A minimum of three replicate test pieces of both treated and untreated materials should be employed.
- vii. The final data should include either some indication of the impact of service conditions on the performance of the treated material / article or data from an ageing study. The intention is to demonstrate how long the claimed benefit will be sustained

Table 4. Added Properties – Example Claims, Problems and Testing Approaches

Claim	Proof Required	Example Method
Bedside cabinet for use in hospitals that has been treated to reduce infections by killing ‘bacteria on contact’.	Data should show that microorganisms, when deposited through skin contact (even under simulated conditions) and through the deposition of fine aerosols are killed within a time-frame that would prevent the surfaces becoming a vector for cross-contamination.	Plaques made of the identical material used for the cabinet are employed in the test. Both treated and untreated variants are used. Simulated cleaning is employed to address durability of the effect.
	Hand (skin) contact	The method described in Figure 11 is employed to deposit MRSA onto test plaques. A range of contact times between 5 minutes and 1 hour are used. A difference of 3 orders of magnitude is suggested to be desirable by medical personnel.
	Aerosol	The method described in Figure 11 is adapted for use by employing multiple droplets of 1 µl on each test plaque. A range of contact times between 5 minutes and 1 hour are used to explore activity. A difference of 3 orders of magnitude is suggested to be desirable by medical personnel.
A plastic conveyer belt is treated to prevent the survival and / or growth of <i>Listeria monocytogenes</i> between cleaning intervals in a food factory.	Data should show that <i>L. monocytogenes</i> grows (or at least survives) on an untreated conveyer belt during a 6 hour interval. No growth and significantly reduced survival should be demonstrated on the treated belt.	Plaques made of the identical material used for the belt are employed in the test. Both treated and untreated variants are used. A simulated cleaning regime is employed to address durability of the effect. ISO 22196 is adapted to simulate a moist conveyer belt. <i>L. monocytogenes</i> is used as the test species and a soiling agent relevant to the end use is included. A contact time and temperature equal to that encountered in practice are employed.

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Appendix A: Methods used to Examine the Resistance of Porous Materials to Biodeterioration: Textiles

Reference	Title	Description	Major Principle / Use
EN 14119:2003	Testing of textiles –Evaluation of the action of microfungi	The test is designed to determine the susceptibility of textiles to fungal growth. Assessment is by visual rating and measurement of tensile strength.	Agar plate test
AATCC 30-2013	Antifungal activity, Assessment on textile materials: mildew and rot resistance of textile materials	The two purposes of the test are to determine the susceptibility of textiles to microfungi and to evaluate the efficacy of fungicides on textiles	Agar plate test
DIN 53931	Testing of textiles; determination of resistance of textiles to mildew; growth test	The test determines the efficacy of treatments for prevention of fungal growth on / in textiles. It also allows the performance testing of a treatment after UV irradiation , leaching <i>etc.</i>	Agar plate test
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of that material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 6085 :1992	Determination of the resistance of textiles to microbial deterioration	The purpose of the method is to assess the extent to which a material will support fungal / bacterial growth and how performance of the material is affected by such growth. Visual Assessment and measurement of tensile strength	a) soil burial test; b) agar plate test, c) humid chamber test
EN ISO 11721-1 (2001)	Textiles - Determination of resistance of cellulose-containing textiles to micro-organisms - Soil burial test- Part 1: Assessment of rot retarding finishing	The test is designed to determine the susceptibility of cellulose containing textiles against deterioration by soil microorganisms. Preserved and unpreserved textiles are compared. Visual Assessment and measurement of tensile strength	Soil burial test
EN ISO 11721-2 (2003)	Textiles - Determination of resistance of cellulose-containing textiles to micro-organisms - Soil burial test- Part 2: Identification of long-term resistance of a rot retardant finish	The test identifies the long-term resistance of a rot-retardant finish against the attack of soil inhabiting microorganisms. It allows to make a distinction between regular long-term resistance and increased long-term resistance. Visual Assessment and measurement of tensile strength	Soil burial test
BS 2011 : Part 2.1J (IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards colonization by fungi.	Humid chamber test (90 to 99% humidity)

AS 1157.2 - 1998	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 1- Resistance to Surface Mould Growth.	Test specimens are inoculated with a suspension of spores of <i>Aspergillus niger</i> and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Specimens are examined for the presence of surface mould growth.	Agar plate test
AS 1157.4 - 1998	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Textiles to Fungal Growth. Section 2 - Resistance to Cellulolytic Fungi.	Test specimens are inoculated with a suspension of spores of <i>Chaetomium globosum</i> and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.3 - 1998	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 2: Resistance of Cordage and Yarns to Fungal Growth.	Test specimens are inoculated with a suspension of spores of <i>Chaetomium globosum</i> and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test.	Agar plate test (other vessels containing media are employed for large specimens).

Methods used to Examine the Resistance to Biodeterioration: Geotextiles

Reference	Title	Description	Major Principle
EN 12225:2000	Geotextiles and Geotextiles-related products - Method for determining the microbiological resistance by a soil burial test	The test is designed to determine the susceptibility of geotextiles and related products to deterioration by soil microorganisms. Visual Assessment and measurement of tensile strength	Soil burial test

Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Paper etc.

Reference	Title	Description	Major Principle
DIN EN 1104 - 05	Paper and board intended to come into contact with foodstuffs Determination of transfer of antimicrobial constituents	A minimum of 20 replicates sub-samples (each 10 - 15 mm in diameter) taken from 10 samples of a batch of paper are placed in intimate contact with nutrient agar plates inoculated with either <i>Bacillus subtilis</i> or <i>Aspergillus niger</i> and incubated at 30°C for 7 days and at 25°C for 8 - 10 days respectively.	Zone Diffusion Assay.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Direct Inoculation	Replicate samples (3) are inoculated with a suspension of fungal spores and then incubated on the surface of a minimal mineral salt medium to determine if they support fungal growth.	Biodeterioration Test.
ASTM D 2020-03	Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard - Soil Burial	Replicate samples (5) are buried in soil for 14 days and then examined for the deterioration compared with unburied samples for both physical deterioration and loss of tensile strength.	Biodeterioration / Biodegradation Test.
AS 1157.7 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Papers and Paper Products to Fungal Growth.	Test specimens are placed on the surface of a mineral salt based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test
AS 1157.5 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 5: Resistance of Timber to Fungal Growth.	Test specimens are placed on the surface of a mineral salt based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Growth on the specimen is assessed.	Agar plate test
AS 1157.6 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 6: Resistance of Leather and Wet 'Blue' Hides to Fungal Growth.	Test specimens are placed on the surface of a mineral salt based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Growth on specimens is assessed. Sucrose containing media is employed where true controls cannot be obtained.	Agar plate test

Methods used to Examine the Resistance to Biodeterioration: Plastics

Reference	Title	Description	Major Principle
ASTM D 5338 - 15	Standard test method for Determining aerobic biodegradation of plastic materials under controlled composting conditions	Test which measures metabolisation rate of plastic materials in compost by measuring CO ₂ output	Biodegradability test
ASTM E 1428 – 15a	Standard test method for evaluating the performance of antimicrobials in or on polymeric solids against staining by <i>Streptovorticillium reticulum</i> (a pink stain organism)	The test shows the susceptibility of solid polymeric materials towards staining. After incubation the test species are rated visually.	Agar plate test
ASTM G 22 - 96	Standard practice for determining resistance of plastics to bacteria	Test designed to determine the effect of bacteria on the properties of plastics	Agar plate test
ASTM G 21 - 15	Standard practice for determining resistance of synthetic polymeric materials to fungi	The method is designed to assess the susceptibility of a material to fungal growth. Rate of growth on the specimen is assessed visually.	Agar plate test
ASTM G 29 - 10	Standard practice for determining algal resistance of plastic films	Test to determine the susceptibility of immersed plastic films to the attachment and proliferation of surface-growing algae	Biofouling test
EN 14047:2003-3	Packaging - Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium - Method by analysis of evolved carbon dioxide	Test which measures metabolisation rate of immersed plastic by measuring CO ₂ output	Biodegradability test
EN 14048:2002	Packaging - Determination of the ultimate aerobic biodegradability of packaging materials in an aqueous medium -Method by measuring the oxygen demand in a closed respirometer	Test which measures metabolisation rate of immersed plastic by measuring O ₂ output	Biodegradability test
ISO 846:1997	Plastics - Evaluation of the action of microorganisms	Method for determining the biodeterioration of plastics due to the action of fungi, bacteria and soil microorganisms. Petri dish tests are performed with or without additional carbon source	Agar plate test; soil burial test
EUROCAE ED-14B / RTCA DO 160B	Environmental conditions and test procedures for airborne equipment; Section 13: Fungus resistance	Mould growth test to show the susceptibility of a material towards the colonization by fungi.	Humid chamber test (90 to 99% humidity)

Methods used to Examine the Resistance to Biodeterioration: Plastics

Reference	Title	Description	Major Principle
MIL-STD-810F	Environmental Engineering considerations and laboratory tests; Method 508.5 FUNGUS	The purpose of the method is to assess the extent to which a material will support fungal growth and how performance of the material is affected by such growth.	Humid chamber test (90 to 99% humidity)
BS 2011 : Part 2.1J (identical with IEC 68-2-10)	Basic environmental testing procedures	Mould growth test to show the susceptibility of a material towards the colonization by fungi.	Humid chamber test (90 to 99% humidity)
ISO 16869:2008	Plastics - Assessment of the effectiveness of fungistatic compounds in plastics formulations	A specimen is placed on a nutrient-salt- agar (without additional carbon source) in a petri dish and overlaid with the same agar containing fungal spores. Rate of growth on the specimen is visually assessed.	Agar plate test
AS 1157.4 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 4: Resistance of Coated Fabrics and Electronic Boards to Fungal Growth.	Test specimens are inoculated with a suspension of spores of <i>Chaetomium globosum</i> and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined and exposed samples are subjected to a tensile strength test. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test

Methods used to Examine the Resistance to Biodeterioration: Plastics

Reference	Title	Description	Major Principle
AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 1: Resistance to Growth	Test specimens are inoculated with a suspension of spores of a range of fungi and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary.	Agar plate test
AS 1157.11 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 11: Resistance of Rubbers and Plastics to Surface Fungal Growth - Section 2: Fungistatic Properties	Test specimens are placed on the surface of a sucrose, mineral salts based agar and then both the specimen and the agar are inoculated with a suspension of spores of a range of fungi. They are then incubated for 14 days and then assessed for growth. Both leached and unleached specimens are examined. Glass rings are employed to hold the specimens in intimate contact with agar when necessary. Growth on both the specimen and inhibition of growth on the surrounding agar are assessed.	Agar plate test

Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Surface Coatings & Adhesives

Reference	Title	Description	Major Principle
BS3900 Part G6	Assessment of resistance to fungal growth	Replicate test panels coated with the test coating are inoculate with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth ($23 \pm 2^\circ\text{C}$ and high humidity / surface condensation). In the published standard, condensation on the test panels is achieved by increasing the temperature in a water bath below the samples for short periods of time. Revisions are in progress that may obviate this step. The method is validated by the need for fungal growth / germination of spores to be observed on a standard coating known to be susceptible to fungal growth after incubation for 2 weeks. After incubation growth is rated in accordance with a scale related to the percentage cover with fungal growth (following visual and microscopical examination). A natural and artificial soiling are described in the method which can be employed when appropriate.	Biodeterioration Test
ASTM D3273-12	Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth.	Biodeterioration Test
WK4201	Standard Test Method for Resistance to Mold Growth on Building Products in an Environmental Chamber	Replicate test panels coated with the test coating are inoculated with a suspension of spores of fungi known to grow on the surface of paints and related materials. The samples are then incubated under conditions suitable to support fungal growth.	Biodeterioration Test
ASTM D5590-94	Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay		Agar Plate Test

Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Surface Coatings & Adhesives

Reference	Title	Description	Major Principle
SS345 Appendix B	Formal Title Missing at Present	The bottom of glass petri dishes are coated with paint. After drying a culture of algae in a suitable growth liquid medium is placed into the dish and incubated under conditions suitable for algal growth.	Biodeterioration Test.
EN 15457:2014	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against fungi	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of 4 fungal species selected from a list of 10. The plates are then incubated at 24°C for X days and then assessed for growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPD. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay
AS 1157.10 - 1999	Australian Standard - Methods of Testing Materials for Resistance to Fungal Growth Part 10: Resistance of Dried or Cured Adhesives to Fungal Growth	Test materials coated onto glass microscope slides are inoculated with a suspension of spores of a range of fungal species and then incubated on the surface of a mineral salts based agar for 14 days and then assessed for growth.	Agar plate test
EN 15458:2014	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against algae	Coatings are applied to glass fibre discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of 3 algal species selected from a list of 5. The plates are then incubated at 23°C under illumination (16 hour day length, 1000 lux) for X days and then assessed for growth using a rating scale. The test is intended to support claims that a biocide can have an effect in a surface coating in support of its listing in the relevant use category within the EU BPR. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay

Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Surface Coatings & Adhesives

Reference	Title	Description	Major Principle
VdL RL06	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of spores of <i>A. niger</i> and <i>Penicillium funiculosum</i> . The plates are then incubated at 28°C for 3 weeks and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and ‘wet’ applications are leached in water prior to testing.	Zone Diffusion Assay / Humid Chamber Test
VdL RL07	Guideline to Evaluate the Resistance of Coating Materials against Mold Growth	Coatings are applied to paper discs and then placed in intimate contact with the surface of nutrient agar plates. The coatings and surrounding media are then inoculated with a mixed suspension of <i>Scenedesmus vacuolaris</i> and <i>Stichococcus bacillaris</i> . The plates are then incubated at 23°C for 3 weeks under illumination (16 hour day length, 1000 lux) and assessed for growth using a rating scale after 1, 2 and 3 weeks. Coatings for exterior use and ‘wet’ applications are leached in water prior to testing.	Zone Diffusion Assay / Humid Chamber Test

Appendix B: Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ASTM E2149-13a	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of <i>Klebsiella pneumoniae</i> and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	Relies on either diffusion of antimicrobial from treated material into the cell suspension. Some activity may be due to interaction between the population and the surface of the material in suspension. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 147-2011	Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method	Agar plates are inoculated with 5 parallel streaks (60 mm long) of either <i>Staphylococcus aureus</i> or <i>K. pneumoniae</i> . A textile sample is then placed over the streaks and in intimate contact with the surface of the agar and incubated. Activity is assessed based on either the mean zone of inhibition over the 5 streaks or the absence of growth behind the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 100-2012	Antibacterial Finishes on Textile Materials: Assessment of.	Replicate samples (sufficient to absorb 1 ml of test inoculum) of fabric are inoculated with individual bacterial species (e.g. <i>S. aureus</i> and <i>K. pneumoniae</i>) suspended in a nutrient medium. The samples are incubated under humid conditions at 37°C for a specified contact time. Activity is assessed by comparing the size of the initial population with that present following incubation. A neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes-Étoffes et surfaces polymériques à propriétésantibactériennes-Caractérisation et mesure de l'activitéantibactérienne	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either <i>S. aureus</i> or <i>K. pneumoniae</i> using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	Cell suspension intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.

Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Qualitative Test	Three replicate samples of fabric, yarn or pile / wadding are placed in intimate contact with the surface of agar plates that have been inoculated with a cell suspension of either <i>S. aureus</i> or <i>K. pneumoniae</i> and incubated at 37°C for 24 - 48 hours. The presence of and size of any zone of inhibition around the samples is then recorded.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
JIS L 1902: 2008	Testing Method for Antibacterial Activity of Textiles Quantitative Test	Replicate samples of fabric (6 of the control and 3 of the treated) are inoculated with individual bacterial species (e.g. <i>S. aureus</i> and <i>K. pneumoniae</i>) suspended in a heavily diluted nutrient medium. The samples are incubated under humid conditions at 37°C for a specified contact time. Activity is assessed by comparing the size of the initial population in the control with that present following incubation. No neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
EN ISO 20645 - 2004	Textile Fabrics - Determination of the antibacterial activity - Agar plate test (ISO/FDIS 20645:2004)	Four replicate samples of fabric (25 ± 5 mm) are placed in intimated contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either <i>S. aureus</i> , <i>Escherichia coli</i> or <i>K. pneumoniae</i> . The plates are then incubated for between 18 and 24 hours and the plates are then assessed for growth based on either the presence of a zone of inhibition of > 1 mm or the absence / strength of the growth in the media overlaying the test specimen.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN 195920	Examination of the Antibacterial Effect of Impregnated Textiles by the Agar Diffusion Method	Four replicate samples of fabric (25 ± 5 mm) are placed in intimated contact with a solid nutrient medium in a petri dish. The samples are then overlaid with molten solid nutrient media which has been inoculated with a cell suspension of either <i>S. aureus</i> or <i>E. coli</i> . The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in BS EN ISO 20645 above.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
SN195924	Textile Fabrics - Determination of the Antibacterial Activity: Germ Count Method	Fifteen replicate samples (each replicate is comprised of sufficient specimens of 25 ± 5 mm to absorb 1 ml of test inoculum) are inoculated with cells of either <i>E. coli</i> or <i>S. aureus</i> suspended in a liquid nutrient medium and incubated in sealed bottles for up to 24 hours at 27°C. After 0, 6 and 24 hours, 5 replicate samples are analysed for the size of the viable population present. A neutraliser is employed. An increase of 2 orders of magnitude of the population exposed to a control sample is required to validate the test. The method defines a textile as antibacterial if no more than a specified minimum level of growth is observed after 24 hours in 4 of the 5 replicate groups of samples.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
SN195921	Textile Fabrics - Determination of Antimycotic Activity: Agar Diffusion Plate Test	Replicate (4) samples of sterilised fabric (25 ± 5 mm diameter) are placed in intimated contact with a solid nutrient medium in a petri dish. Each petri dish has been prepared as a double layer. The first layer consists of 10 ml nutrient agar, the second layer of another 10 ml of the same nutrient agar to which 0.1 ml spore suspension (10^7 ml ⁻¹) of either <i>Candida albicans</i> , <i>Aspergillus niger</i> , <i>Cladosporium sphaerospermum</i> or <i>Trichophyton mentagrophytes</i> had been added. The plates are then incubated at 28 °C either 2 days (<i>C. albicans</i>) or 7 days (<i>A. niger</i> , <i>C. sphaerospermum</i> and <i>T. mentagrophytes</i>). The test is valid when control specimens of the same material without biocide, or of a biocide-free standard specified cotton material are fully overgrown. Good antimycotic efficacy is considered to be demonstrated when the specimens show no fungal growth on their surface. The test specifies that both sides of a material have to be tested.	Zone diffusion assay. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Absorption method	Replicate (6) samples of textile are inoculated with a standardised broth culture of either <i>S. aureus</i> or <i>K. pneumoniae</i> in individual tubes and then incubated at 37°C for 18 - 24 hours in closed containers. Samples are analysed for the presence of viable bacteria both before and after incubation by either total viable count or the determination of total ATP. Samples are sterilised prior to testing and a neutraliser is employed during recovery. The test is validated by growth of \square 1 order of magnitude during the incubation period.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Transfer method	Replicate (6) samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either <i>S. aureus</i> and <i>K. pneumoniae</i> using a 200g weight for 1 minute. The samples are then removed. Replicate (3) samples are analysed for the either the number of viable bacteria or the total ATP content both before and after incubation under humid conditions at 37°C for 24 hours. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either growth of \geq 1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	Replicate (6) samples of test material are either <i>S. aureus</i> and <i>K. pneumoniae</i> by 'printing' cells collected on a membrane filter onto their surface in a standardised manner. The samples are then incubated under humid conditions for 18 - 24 hours at 20°C for a specified contact time(s). Replicate (3) samples are analysed for the either the number of viable bacteria or the total ATP content both before and after incubation. Samples are sterilised prior to testing and a neutraliser is employed during cell recovery. The test is validated by either determining the survival of the inoculum on the control material.	'Dry' inoculum intimate contact test. The transfer method of inoculation could be adapted to provide some simulation data.

Methods used to Examine the Antimicrobial Activity of Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ISO 13629-1	Textiles - Determination of Antifungal Activity of Textile Products: Part 1 - Luminescence Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the ATP concentration associated with the samples. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced concentrations of ATP associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.
ISO 13629-2	Textiles - Determination of Antifungal Activity of Textile Products: Part 2 - Plate Count Method	Samples of textiles are inoculated with a suspension of fungal spores either by direct application or transfer from an agar surface and then incubated. Germination and growth of the spores is followed by measuring the number of colony forming units. The presence of an antifungal treatment is expected to show either an inhibition of germination or a reduction in the rate of growth as indicated by reduced numbers of colony forming units associated with the treated material in comparison with the untreated material.	Basic efficacy test that has limited use as a simulation of final use of a treated material. The transfer method of inoculation could be adapted to provide some simulation data.

Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Qualitative Antibacterial Activity	Petri dishes with nutrient media are inoculated with a single, diagonal streak (approx.7.5 cm) of either <i>S. aureus</i> or <i>K. pneumoniae</i> . An unsterilized test specimen (25 mm x 50 mm) is placed in intimate contact and transversely across the inoculum on the agar surface. The plates are then inoculated at 37°C for 18 - 24 hours. The front and back of the carpet are tested separately. After incubation, the plates are inspected for the presence of growth, both below the specimens and for any zone of inhibition caused by the specimen is recorded. The test can also be used to test the effect of cleaning regimes. An untreated control is optional.	Qualitative assessment of rate of kill and zone diffusion test Basic efficacy test that has limited use as a simulation of final use of a treated material.
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antibacterial Activity	Unsterilized specimens of carpet are pre-wetted with either sterile water or a wetting agent before being inoculated with individual suspensions of either <i>S. aureus</i> or <i>K. pneumonia</i> in either a low or a high nutrient solution. The samples are then incubated in a tightly closed jar at 37°C for a specified contact time. Cells are recovered in 100 ml of a neutraliser after 0 and 6 - 24 hours of incubation. Activity is assessed by comparing the size of the initial population in the control (if used) with that present following incubation. A control is optional. When not employed, viable counts following incubation of the treated specimens alone are considered. The test can also be used to test the effect of cleaning regimes.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Methods used to Examine the Antimicrobial Activity of Carpets

Reference	Title	Description	Major Principle
AATCC 174-2011	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabaroud Dextrose Agar are inoculated with 1 ml of a spore suspension of <i>Aspergillus niger</i> . Immediately afterwards, specimens (38 mm diameter) of unsterile test material are placed into intimate contact with the agar. An additional 0.2 ml of the same spore suspension is also employed to inoculate the test pieces directly. The samples are then incubated at 28°C for 7 days. The back and front of the discs of carpet are tested in separate dishes. The zone of inhibition and the growth of fungus on the upper surface of the specimens are reported (no growth, microscopic growth, macroscopic growth). The test can also be used to test the effect of cleaning regimes.	Zone diffusion test / surface growth test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
WIRA Test F	Test Method for Assessing the Survival of Test Organisms on Floor Coverings	Specimens (850 mm x 350 mm) are conditioned at 20°C and 65% RH before being subjected to 2 wet and 2 dry passes using a commercial spray extraction machine or a test rig. After 24 h drying, 12 specimens (each 60 mm diameter) are cut from the carpet. An aliquot (1 ml) of a suspension of cells of <i>E. coli</i> in nutrient broth is poured onto filter paper (7 cm diameter). The filter paper is then pressed for 1 min onto the surface of the carpet using a 1 kg weight. The filter paper is then discarded. After 0, 6 and 24 hours incubation at a specified temperature the carpet's surface is pressed onto contact plates of McConkey Agar. After 24h replicate (3) plugs (10 mm) are taken from each specimen and suspended in 10 ml nutrient broth for 30 seconds and then analysed for the presence of <i>E. coli</i> by total viable count.	Cell suspension intimate contact test. Potential to demonstrate the effectiveness of an antimicrobial treatment if appropriate incubation conditions are selected and addition species employed.

Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2010	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate samples (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either <i>E. coli</i> or <i>S. aureus</i> in a highly diluted nutrient broth. The cell suspension is then held in intimate contact with the surface by the use of a sterile polyethylene film (usually 40 mm x 40 mm) for 24 hours at 35°C under humid conditions. The size of the population on the treated surface is then compared with the size on the control surface both prior to and after incubation. A neutraliser for certain biocide types is employed. Antibacterial activity is certified if the difference between the Log10 of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
ISO 22196:2011	Plastics - Measurement of antibacterial activity on plastics and other non-porous surfaces.	This ISO norm was created from JIS Z 2801 by the SIAA of Japan in collaboration with the IBRG (see above). No performance criteria are included and its scope was extended in 2011 to include all non-porous materials	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.
XP G 39-010	Propriétés des étoffes-Étoffes et surfaces polymériques à propriétésantibactériennes-Caractérisation et mesure de l'activitéantibactérienne	Four replicate samples of test material are placed in contact with an agar plate that has been inoculated with a specified volume of a known cell suspension of either <i>S. aureus</i> or <i>K. pneumoniae</i> using a 200g weight for 1 minute. The samples are then removed. Duplicate samples are analysed for the number of viable bacteria both before and after incubation under humid conditions at 37°C for 24 hours. A neutraliser is employed during cell recovery.	Cell suspension intimate contact test. Basic efficacy test that has limited use as a simulation of final use of a treated material.

Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
ASTM E2180-12	Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials	Replicate (3) samples of material are inoculated with cells of either <i>S. aureus</i> or <i>K. pneumoniae</i> suspended in molten semi-solid isotonic saline / agar. This attempts to form an 'artificial biofilm' which holds the suspension in intimate contact with the test surface of inherently hydrophobic materials. Samples are then incubated at a temperature similar to that intended for the final use for a specified period (usually 24 hours) under humid conditions. The size of the viable bacterial populations on the control and treated surfaces is then determined using a dilution plate count. Any effect is recorded using percentage reduction calculated from the geometric means of the data. A neutraliser may be employed and sonication is used to separate the 'biofilm' from the test surfaces and suspend the agar gel. Subsequent imprinting of the test surface onto solid nutrient media can be performed to look for the presence of adherent viable cells.	<p>Immobilised cell suspension intimate contact test.</p> <p>Basic efficacy test that has limited use as a simulation of final use of a treated material.</p>
ASTM E2149-13a	Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions	Dynamic shake flask test. Test material is suspended in a buffer solution containing a known number of cells of <i>Klebsiella pneumoniae</i> and agitated. Efficacy is determined by comparing the size of the population both before and after a specified contact time.	<p>Relies on either diffusion of antimicrobial agents from treated material into the cell suspension or due to interaction between the population and the surface of the material in suspension.</p> <p>Basic efficacy test that has limited use as a simulation of final use of a treated material.</p>

Appendix C: The Role of Mic Data in Supporting Claims for Treated Materials

Although Minimum Inhibitory Concentration (MIC) data may not have a direct role in supporting claims and demonstrating the benefit of treated materials and treated articles, they can provide useful supporting information. For example, a treated material may have been shown to demonstrate effectiveness in reducing the viability of model Gram Positive and Gram Negative bacteria (such as *E. coli* and *S. aureus*) when delivered to a surface as a splash of contaminated liquid. MIC data may be available from the manufacturer of the active substance employed that demonstrates activity against a wide range of other bacteria at a similar level of sensitivity to the model species used in the efficacy simulation. It might be anticipated therefore that, provided that these species are morphologically and physiologically similar (i.e. they are not obligate anaerobes or species that produce endospores that can survive exposure), they will respond in a similar manner when exposed to the material under more realistic conditions. Caution needs to be taken when applying such a principle, and where a species is critical to a specific claim it should be employed in the efficacy test. However, MIC data can provide useful supporting information.