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ANALYSIS AND ASSESSMENT OF CURRENT PROTOCOLS TO DEVELOP HARMONISED TEST METHODS AND RELEVANT PERFORMANCE STANDARDS FOR THE EFFICACY TESTING OF TREATED ARTICLES / TREATED MATERIALS

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Series on Pesticides

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ANALYSIS AND ASSESSMENT OF CURRENT PROTOCOLS TO DEVELOP HARMONISED TEST METHODS AND RELEVANT PERFORMANCE STANDARDS FOR THE EFFICACY TESTING OF TREATED ARTICLES / TREATED MATERIALS

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The Pesticide Programme was created in 1992 within the OECD's Environmental Health and Safety Division to help OECD countries:

- harmonise their pesticide review procedures,
- share the work of evaluating pesticides, and
- reduce risks associated with pesticide use.

The Pesticide Programme is directed by the Working Group on Pesticides, composed primarily of delegates from OECD Member countries, but also including representatives from the European Commission and other international organisations (*e.g.* United Nations Food and Agriculture Organization, United Nations Environment Programme, World Health Organization, Council of Europe), and observers from the pesticide industry and public interest organisations (NGOs).

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The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, ILO, FAO, WHO, UNIDO and the OECD (the Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. UNITAR joined the IOMC in 1997 to become the seventh Participating Organization. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

Background

The "OECD Efficacy Workshop on Certain Antimicrobial Biocides" was held April 22-24, 2002 in Washington, D.C. and was attended by experts from government, academia and industry.

A Steering group was formed at the Workshop to carry forward the recommendations of the participants to investigate the potential for harmonisation of test methods for antimicrobial biocides used in treated articles/materials.

An investigation was therefore conducted to analyse and assess current protocols to develop harmonised test methods and performance standards for the efficacy testing of biocides used in treated articles. The present report is the result of this investigation.

How this document was developed

The document was produced by Mr. Peter Askew from Industrial Microbiological Services Ltd, and overseen by the OECD Task Force on Biocides' Steering group on treated articles.

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

Executive Summary

Antimicrobial activity has been a component of a wide range of materials for many years for example, being used to prevent fungal biodeterioration of plastics used for the insulation of electrical cables. In the last few years this activity has been extended to provide a wider spectrum of activity and materials are now being produced which are intended to provide hygienic benefits through their use. While many methods exist for investigating both the susceptibility of materials to microbial spoilage and the efficacy of treatments intended to limit such spoilage, few have been designed to explore hygienic benefits. During an OECD workshop IN April 2002 on the efficacy of certain antimicrobial biocides, the need for a harmonised approach to assessing the claims made for these emergent applications was identified. It was recognised that this approach should encompass claims for both biocidal and biostatic activity and all relevant types of microorganisms. A hierarchy of tests was proposed with initial focus being on the demonstration of basic activity. It was clear that these tests would need to produce data that was both scientifically and statistically valid and it was considered important that this data should be fully quantitative.

Treated articles encompass a wide range of materials from textiles to powder coated steel. The claims made for these materials are equally diverse. However, at the most basic level the materials can be divided into 2 broad categories depending on whether the finished article is either porous (*eg* most textiles, paper) or non-porous (*eg* plastics, surface coatings). Similarly, the effects claimed essentially fall into 2 categories depending on whether the overall effect is intended to be either biocidal or biostatic. Unlike most disinfectant applications, the size of the effect will be related to the claim made for the article. An arbitrary measure of performance (*eg* reduction by 5 orders of magnitude following contact for 5 minutes) is not considered useful for treated articles. The purpose of the testing methodology is purely to provide a mechanism to demonstrate that any effect claimed can be demonstrated in a scientifically and statistically significant manner.

A review of methods that examine the relationship between microorganisms and a wide range of materials (*eg* textiles, plastics, coatings *etc*), either currently available or in an advanced stage of development was performed. Two approaches were identified which appear to be capable of satisfying the requirements of a basic efficacy test (Tier 1) for treated articles.

It is considered that the approach typified by that described in AATCC 100: 1998 could be adapted to quantify the biocidal and biostatic properties of porous treated articles.

It is considered that the approach typified by that described in JIS Z 2801: 2000 could be adapted to quantify the biocidal and biostatic properties of non-porous treated articles.

Neither method is considered suitable for assessing the impact of treated articles on the growth of either filamentous fungi or algae however, a wide range of national and international standards exist which are. Both methods would require some modification to examine biostatic properties and data would be required to extend the knowledge on both variability and the constraints on the

size of effect that could be measured. Methods based on both are present in current draft ISO standards and are widely used in industry and so little resistance to their forming the basis of a harmonised approach is anticipated.

While the methods mentioned above are also capable of generating data relevant to certain in-use situations where free water is present at least for part of the duty cycle, further modifications and the development of additional protocols will almost certainly be required to provide data for more application specific claims (Tier 2 and 3). Methods are under development to examine the efficacy of treated articles under conditions where no / minimal free water is present but are far from robust at present. Full validation of such methods through the use of international ring tests will be required. Some form of correlation of both these and more application specific tests with performance in the field should be considered. The benefit of using treated articles will need to be reviewed, possibly on an application by application basis (*eg* can the use of treated articles have a significant impact on hospital acquired infections?) and data on any negative impact on their use will be required (*eg* does their use have any impact on the resistance characteristics of microorganisms with clinical significance?). Finally, it would be useful if the terminology used to describe treated articles was harmonised. The use of treated material as a generic term and treated article as a specific term is discussed.

CONTENTS

1 Introduction

Many methods exist worldwide for examining the relationship between microorganisms and both man-made and natural materials. Much of the emphasis of these methods is related to the spoilage, deterioration or defacement of materials whether these be foodstuffs, structural woodwork or water based coatings (Ref 1). Most of the testing technology is focussed on determining both the susceptibility of materials to microbial attack and to the efficacy of agents intended to either prevent or limit this attack. In many cases, these tests are used to form the basis of claims about how well a certain material, additive or technology may be expected to perform when exposed to microbiological challenges. Often this information is used to make commercial comparisons between either different final products or additives as well as to attempt to predict whether a material will comply with a certain specification (*eg* service life).

1.1 Antimicrobial Agents

Much of the technology mentioned above depends on the use of antimicrobial agents to prevent growth in association with the material to be protected. In other disciplines, antimicrobial agents are employed to either limit the growth of or kill microorganisms within a process, possibly to prevent them impacting on materials they may come into contact with. A good example of such antimicrobial agents are the additives employed in the water treatment industry. These agents are used in applications such as cooling and humidification systems and paper mills (Ref 1). They are introduced to both eliminate health risks associated with the uncontrolled growth of microorganisms (*eg* prevention of the growth of *Legionella spp* in calorifiers) and limit the impact that they may have on structural components within the process (*eg* corrosion, loss of heat transfer efficiency) and the products of the process (*eg* foul odour in air handling systems, defects in paper resulting from bacterial slimes).

Antimicrobial agents are also employed to remove microbial populations from either within the matrix of a material or on the surfaces of a material. These agents are often applied as washes or rinses and are used to either sterilise / disinfect or at least reduce either part or all of any microbial population that may be present (Ref 2). Such disinfection processes can also take the form of an addition of an antimicrobial agent to a matrix which contains a microbial population (*eg* a contaminated metal working lubricoolant). Often this will be combined with the introduction of protection against further growth (Ref 3).

Finally, antimicrobial agents have a long history of use in human and veterinary medicine, crop protection and horticulture. Here they are either introduced as topical applications or in a systemic manner. The intention again being either to 'disinfect' (*eg* the use of oral antibiotics in humans), protect (*eg* the prophylactic use of certain veterinary drugs and crop protection fungicides) or modify (*eg* improvement of yield in dairy herds through modification of the microflora of the rumen by antibiotics) the system to which they are applied.

1.2 Treated Articles / Treated Materials

1.2.1 Definitions

Following a workshop in April 2002 entitled "OECD Efficacy Workshop on Certain Antimicrobial Biocides", a draft definition for treated articles / materials was produced (Ref 4). This defined these substances as "a plastic, textile or other pre-formed articles pre-treated with products before first use. The intended benefit is either to preserve the integrity (chemical or physical) and / or aesthetics of such material / article and / or reduce risk of microbial infection". It was noted that the preservation of the treated article itself was not considered to be within the scope of this definition. Given this limitation, and the apparent contradiction with the intention to preserve integrity / aesthetics of the article / material the following considerations have been made when examining test methods suitable to support claims from treated articles / materials.

Clearly, in a number of the situations described in Section 1.1 above, materials and articles made from those materials can be seen to have been treated with an antimicrobial agent to obtain some form of microbiological effect. For example, plasticised poly vinyl chloride (PVC) may be formulated with the addition of a fungicide to defend it from attack by microfungi and so protect the plasticiser and prevent loss of elasticity in service (Ref 5). Similarly, water used in the production of water based paints may have been treated to prevent the introduction of microorganisms into both the production facility and the final product, thus maximising the efficacy of any in-can preservatives employed in the formulation. A food production surface might also be treated with a disinfectant formulation to remove a population prior to use. This disinfectant might even be carried within the matrix of a single use disinfectant wipe manufactured from a non-woven textile.

In most of the situations described above, the treatment of a material / article is intended to either prevent microbiological deterioration of that article, maximise the protection of the material or remove a microbial population from a system prior to its use. However, in recent years a new form of interaction between a formulated material and microbial populations has emerged (Ref 6). In part, this can be viewed as either an extension of the degree of protection provided to a material by the inclusion of an antimicrobial agent into it or as the transfer of the properties of external treatments of a material into the material itself. The inclusion of the antimicrobial agent is now not simply to protect the material from deterioration but to exert a biological effect either to the immediate surrounding of that material or to items that come into contact with it. These effects may range from the prevention of growth of undesirable microbial populations on a material to which they pose no physical, chemical or biological threat to the immediate destruction of individual microbial cells as they come into close association with a surface (possibly without even coming into direct physical contact). In all cases the effect is external to the material from which the article is constructed and is not merely present to protect either the material or the article itself. However, it is possible that the effect may take place within an article constructed from a modified / treated material. For example, it is possible to imagine an

air filter constructed of paper into which antimicrobial properties have been introduced which is intended to kill bacteria pathogenic to man which impact on it (Ref 7). Similarly, a polyethylene foam sponge may be impregnated with an antimicrobial agent which is intended to prevent the growth of bacteria associated with food poisoning in man. This sponge may not be intended to disinfect surfaces on which it is used but simply to prevent it from becoming a reservoir of such bacteria in a food preparation environment. In contrast, a hand dish-washing liquid which includes an antimicrobial agent in addition to that required to protect it from spoilage and which is intended to be applied to an unmodified polyethylene sponge to prevent the growth of bacterial associated with food poisoning is not necessarily transforming that sponge into a treated article. It can be considered to be acting either as a preservative to any water retained in the sponge or as a disinfectant for the sponge unless it is designed to modify the material from which the sponge is constructed.

Clearly, there are some complex situations when the effects intended by treated articles / treated materials are to be considered and this will impact on the suitability of the method used to measure them. In general however, the effect of a treated article / treated material can be considered to be external to it. The effect is not concerned with either preservation or protection of the material / article itself and is not achieved by the application of a disinfecting agent after the material has entered service. There may of course be instances where the composition of the treated article has an impact on both its preservation and some external hygienic claim. This will need to be clarified but can probably be differentiated by either the claim made or the intent apparent. For example, a material intended for use in a humid environment (*eg* a domestic shower curtain) may legitimately include a fungicide to protect it from fungal growth in service. However, if a claim is made that preventing mould growth on the material reduces the impact of fungal growth on human health then this might well be regarded as a claim related to a treated article. Although the hygienic effect is incidental to the main purpose, a more rigorous basis for the secondary claim may be required to support it (*ie* duration of effect, spectrum of activity *etc*). Similarly, there are certain technologies (*eg N*-halomines; Ref 8) that achieve their external effect by the release of halogen species. These can be both activated and regenerated by the application of hypochlorous acid in the form of dilute solutions of chlorine based bleach. The functioning of treated articles based on this technology is associated with the application of a recognised disinfectant during service and data supporting claims made of such material would need to reflect this.

In addition to the above, there is also the issue of hygienic / antimicrobial coatings. In theory, these materials can have the effect of transforming an object coated with them into a treated article. For example, a liquid paint may well be formulated with either both an in-can preservative and an additional antimicrobial agent or a high level of an antimicrobial agent that can demonstrate both in-can protection and effects in the film formed. The claim may be that the use of this coating will prevent bacterial growth, kill bacteria on contact, improve hygiene *etc* and clearly this would need to be demonstrated using suitable methodology and an appropriate substrate. There may be instances where a claim is not made but intent is present due to the

technology employed and this will require clarification at the regulatory level.

Finally, when considering suitable test methodologies, the scale and duration of the effect may need to be considered with respect to the claim made. For example, will the material / article be able to demonstrate the effect claimed for the effect to have any realistic benefit? Similarly, will the scale of the effect be sufficient to provide the benefit either claimed or implied? Clearly, it is unlikely that data to support such claims would be available from a single test and it is likely that aging and weathering studies would be needed in addition to tests which provide basic proof of principle and demonstrate performance under conditions which simulate actual use.

1.2.2 Product Types

During the workshop on biocidal products described in reference 4, a number of examples of products which were regarded as treated articles / materials were given (see Table 1). This list is consistent with the examples used in Section 1.2.1 above and can be allocated to a number of product types which will have a direct impact on the type of basic test method needed to measure their performance.

Porous / Absorbent Materials

Both from the products listed in Table 1 and more extensive reviews of the market, a number of materials / articles which have a porous / absorbent nature can be identified. These include:

i woven textiles manufactured from either natural, synthetic or mixed fibres
ii non-woven textiles manufactured from either natural, synthetic or mixed fibres
iii paper, board and natural polymeric materials (*eg* wood, leather)
iv non-porous materials which have been made into absorbent articles through a manufacturing process (*eg* polyethylene foam sponge, carpeting *etc*)

Non-Porous Materials

In general, most treated articles produced from non-porous materials have been produced through the incorporation of an antimicrobial agent into the matrix during the manufacture of the material (*eg* through the addition of an antimicrobial masterbatch during the manufacture of vinyl sheet flooring). However, in some instances the surface of the material is modified either chemically or physically to produce the antimicrobial effect (*eg* the fusion of nanoparticulate TiO_2 onto the surface of glass).

i metal surfaces
ii glass and ceramics
ii rigid, semi-rigid and flexible polymeric materials

iv inherently porous / absorbent materials that have been modified to render them non-porous (*eg* PVC coated textiles)

Coatings

In some instances coatings can be regarded as homologous with the material itself (*eg* UV cured films applied to flexible polymeric substrates, coil coatings on steel *etc*). In some cases they are applied during manufacture of an article (*eg* powder coatings), in others they are applied *in-situ* (*eg* aqueous based wall paints). In all cases however, they provide an effect to the surface of the material coated and transform the functional face of the article into a treated article. Judgement may need to be taken when more than one type of material is laminated with another (*eg* when polystyrene formulated with an antimicrobial agent is laminated / co-extruded with unfortified polystyrene to provide material with one antimicrobial surface).

i coatings applied during manufacture of an article with a durable effect
ii coatings applied after manufacture to transform the substrate into a treated article with a durable effect
ii coatings applied to provide transient antimicrobial properties

1.2.3 Product Claims

During the workshop on biocidal products described in reference 4, a number of examples of claims made for products which were regarded as treated articles / materials were given (see Table 1). As with product types, these can be divided into a number of categories which help clarify the form which data used to support the claims must take. During the workshop referred to above a number of terms were defined which can be utilised to help categorise these claims. In practice it is likely that a broader claim such as 'antimicrobial' may wish to be made but for the purposes of test method definition some sub-division is considered essential at least for most of the *in-vitro* level of testing. A consortium based approach may be suited to simulation testing once basic principles have been established (*eg* formation of biofilms on certain devices).

Biocidal Activity

Biocidal activity against one or more groups of microorganism may be claimed. This will be impacted on by the microbial types /species which are employed in testing and, to a certain extent, the type of test required. At this time the scale of the effect will be disregarded but it will result in a reduction in the number of test microorganisms as a result of an interaction with the material through an irreversible, killing effect.

i Bactericidal the effect is limited to a reduction in the size of a vegetative bacterial population.

ii	Fungicidal	the effect is limited to fungi. This effect may be attributed to activity against vegetative growth, spores / resting structures or both and may require clarification depending on the intended use of the product.
iii	Sporicidal	the effect is against the spores / resting structures of bacteria
iv	Virucidal	the effect is limited to virus particles
v	Protisticidal	the effect is exhibited against protozoa and their resting stages
vi	Algicidal	the effect is exhibited against algae and their resting stages.

Biostatic Activity

Biostatic activity against one or more groups of microorganisms may be claimed. As with biocidal activity, this will be impacted on by the species which are employed in testing and, to a certain extent, the type of test required. In general, the prevention of growth / metabolism of the target species should be demonstrated. It may be sufficient to demonstrate that growth on the treated article / material is either slower or reaches a lower level that on an equivalent control material to substantiate a claim. For example, if the intention of some sports clothing is to reduce the production of odour in service through the inhibition of microbial activity (rather than a masking effect), it may be sufficient to reduce the growth rate of the species responsible for creating odour rather than preventing their growth completely. Clearly, here the important criterion is that the effect demonstrated, substantiates the claim made. It is important to demonstrate that conditions suitable for the growth of the target species have been achieved and ultimately that they are relevant to the application supported. An equivalent subdivision of the type of activity that could be supported by relevant test technology to that described for biocidal activity might be:

i	Bacteriostatic	the effect is limited to the prevention of growth / metabolism of bacteria and possibly the germination of bacterial endospores and other resting structures.
ii	Fungistatic	the effect is limited to the prevention of growth of fungi and possibly the germination of fungal spores and other resting structures.
iii	Algistatic	the effect is limited to the prevention of growth of algae and possibly the germination of resting structures.

It is difficult to envisage a simple scenario in which prevention of the growth of protozoa alone would be required and the assessment of activity against this group would probably need to be assessed within more complex community based studies.

1.2.4 Hierarchy of Test Methodologies

During the workshop on biocidal products described in reference 4, it was recognised that it was unlikely that a single test method could satisfy all of the requirements for data to support claims

made for treated articles / materials. In Sections 1.2.2 and 1.2.3 it has been established that a wide range of product types exist and that a diverse list of potential effects might be claimed. This can be reduced to a number of categories which can be used to help target a test methodology towards an effect on a given article / material however, it would be naive to expect any single test to fully replicate the performance expected of the product under conditions of normal use. Thus, a cascade of tests may be required to fully support / explore the potential activity of any given treated article / material ranging from relatively simple tests to demonstrate proof of principle (Tier 1), through simulation of realistic exposure conditions (Tier 2) to in-use evaluation (Tier 3). The main focus of this report is to identify possible routes for the harmonisation of test protocols for Tier 1 although consideration will be given to the potential requirements of Tier 2 and Tier 3 tests.

Table 1: Examples of Treated Articles / Materials

Product	Type	Claims Made
Antibacterial Fabric	Textile	Effective control and prevention of growth of a wide range of microorganisms
Socks	Textile	Improved freshness, reduces odour, inhibits growth of bacteria
Tights (Hosiery)	Textile	Combats the growth of fungi that cause 'Thrush' and 'Athlete's Foot'.
Bathroom Towel	Textile	Prevents microbial growth and maintains hygiene of towel.
Kitchen Sponge	Polymeric Foam	Reduced growth of bacteria on sponge, helps prevent the spread of disease.
Kitchen / Floor Wipes	Non-woven textile	Hygienic, built-in protection against bacteria.
Lavatory Brush	Rigid / Flexible Polymer	Helps prevent growth of bacteria on the body of the brush.
Flooring	Flexible Polymer	Effective / lifetime antimicrobial protection ensuring that the floor remains free of bacteria between cleaning cycles.
Hygienic Coated Steel	Polymer	Neutralises the ability of bacteria to function, grow and reproduce.
Wall coating	Synthetic Paint	Prevents spread of germs and reduces risk of infection, provides protection against harmful bacteria.
Multi-surface Coating	Polymeric dispersion	Kills microorganisms in contact within 4 hours.

2 Critical Parameters that Impact on the Determination of the Performance of Treated Articles / Treated Materials

During the workshop on biocidal products described in reference 4, a number of parameters considered critical to the measurement of the antimicrobial activity of treated materials / articles were produced (see Table 2). It can be seen that these fall into several categories and a number of these parameters can be considered fundamental. For example, if data is used to support the claim that a treated article prevents the growth of bacteria on its surface, it is obviously essential to demonstrate that bacteria would grow on an equivalent substrate but unmodified substrate under equivalent conditions. The principle of the appropriate control is a key feature. Similarly, it is important that the physical / chemical conditions presented to the test population should be suitable to support the growth / survival of that population. The tests should be capable of accommodating species that are relevant to the claim being made and the data generated should be sufficient that any differences seen can be shown to support the claim being made in a statistically valid manner. The design of the protocol should also be such that inter-laboratory variation is smaller than the overall effect being claimed, *ie* if one laboratory can demonstrate a statistically valid effect from a given article / material, a second laboratory should be able to demonstrate a similar, statistically valid effect. The former constraint requires a sound knowledge of the sources of variation within the test design and the appropriate level of replication while the latter dictates the need for inter-laboratory ring tests as part of the validation process of a test protocol. A standard reference material may be required in some cases.

Many of the parameters listed in Tables 2a and 2b will apply both to a Tier 1 basic efficacy test and to tests in Tier 2 which attempt to simulate real conditions of use. Indeed, some may have relatively little impact on the outcome of a Tier 1 test if the intention is simply to demonstrate that a given treated material / treated article has potential to demonstrate the effect claimed (given that in some cases, even when a sizeable effect can be detected under laboratory conditions that under some conditions in practice this effect could not be realised or it proved to provide no benefit). This will be given further consideration when existing individual relevant test procedures are examined below. Questions regarding duration of effect and the impact of ageing and durability of any effect claimed would almost certainly fall into Tier 2 / 3. Within Table 2 it can be seen that consideration has almost solely been given to tests in which microorganisms suspended in some aqueous medium are exposed to the test surface. It is likely that the interaction between a microorganism and a treated article / material under conditions in which free water is absent will have a significant impact on the effect claimed. This is discussed further, later in this report. Concerns have also been expressed about the impact of treated articles / materials on the tolerance / resistance of microorganisms to both antimicrobial agents used in hygiene related applications and health care. This is probably beyond the scope of tests in Tiers 1 and 2 but may be important for validation of claims and the examination of the impact of an effect under Tier 3.

Table 2a: Critical Test Method Parameters (From OECD Biocide Meeting in Bold)

Category	Parameter	Impact
Test Sample	**Relevant Control / Standard**	Validity of claim / need for effect
	Preparation *eg* Sterilisation, cleaning, ageing	Interference
	Size, weight, shape, surface texture	Interaction of inoculum with surface
	Hydrophobicity / absorbency / stability	
	Number of replicates samples	Measurement of effect
Inoculum	**Range of test organisms**	Relevance to claim
	Selection of strains	
	Maintenance of strains	Vigour of test strains / maintenance of any 'special' characteristics.
	Preparation of inoculum	Vigour / susceptibility of inoculum
	Size of bioburden	Scale of effect required
Exposure Conditions	**Suspension / delivery medium**	Detection of biocidal or biostatic effect. Effect on susceptibility of population to effect.
	Delivery mechanism (spray, drip, dip *etc*)	Relationship of inoculum with surface and vigour of inoculum
	pH of system	Growth / survival of test species & relevance to end use
	Presence of Soiling agents	Effect on inoculum and effect on mechanism of claimed action.
	Exposure temperature	
	Duration of exposure	
	Humidity during exposure	
	Surface area : volume ratio	
	Are conditions static or dynamic?	
Recovery Mechanism	**Recovery fluid**	Effect on inoculum
	Use of Neutraliser	Interaction with effect mechanism
	Volume of recovery medium	Limit of detection / efficacy of recovery
	Method of recovery	Effect on inoculum
	Efficiency of recovery method	Limit of detection and size of effect claimed
	Measurement of recovered population	

Table 2b: Critical Test Method Parameters (From OECD Biocide Meeting in Bold)

Category	Parameter	Impact
Handling of Data	**Validation of initial population**	Validity of test / claim
	Measurement of variation	Measurement of size of effect
	Calculation of effect	Support of claim
	Statistical validity of effect	Validity of claim
	Biological validity of effect	
	Comparison with claim made	

3 Existing Test Methodologies

During the 'Biocides Workshop' referred to above a number of tests were identified. Some of these were relevant to external effects related to treated articles / materials as defined in Section 1, others are aimed at demonstrating either susceptibility to microbial growth or the efficacy of biocides incorporated to minimise biodeterioration of the material. In some cases both functions could be demonstrated. Tables 3 - 8 include the above methods and others which have either been identified or submitted as potentially relevant and are keyed with this in mind.

3.1 Existing Test Methodologies - Porous Surfaces

3.1.1 Textiles

It can be seen from Table 3 that there are 3 major forms of test for microbiological effects of treated textiles which are not related to the prevention of biodeterioration. In the first, typified by part of JIS L1902, samples of textile are placed onto agar plates which have been inoculated with bacteria and then incubated. The intention is that intimate contact between the textile and the bacteria / growth medium will result in the inhibition of growth either immediately adjacent to the textile or in an area around the textile should any antimicrobial agents that have been employed become dissolved in the growth medium. These methods are generally acknowledged as being non-quantitative although they could potentially be employed as assays of certain antimicrobial products in the same manner that such techniques are used for certain antibiotics (Ref 9). This could be useful as a screening tool and for investigating the effect of wash cycles *etc*. These methods are widely employed in the textile industry as they provide a highly graphic representation of antimicrobial activity although this can lead to misunderstanding of either the scale of effect seen (bigger zones of inhibition looking better) and the implications that mobility of active ingredient could have on service life. Although these techniques are considered to be

Table 3a: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ASTM E2149-01	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 *Klebsiella pneumoniae* 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.
AATCC 147-1998	Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method	Agar plates are inoculated with 5 parallel streaks (60 mm long) of either *Staphylococcus aureus* or *K pneumoniae.* 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.
AATCC 100-1999	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 (*eg Staph aureus* and *K pneumoniae*) 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.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	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 *Staph aureus* and *K pneumoniae* 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.

Table 3b: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
JIS L 1902: 1998	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 *Staph aureus* or *K pneumoniae* 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.
JIS L 1902: 1998	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 (*eg Staph aureus* and *K pneumoniae*) 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.
prEN ISO 20645	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 *Staph aureus*, *Escherichia coli* or *K pneumoniae*. 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
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 *Staph aureus* or *E coli*. The plates are then incubated for between 18 and 24 hours and the plates are then assessed as described in prEN ISO 20645 above.	Zone diffusion assay

Table 3c: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: 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 *E coli* or *Staph aureus* suspended in a liquid nutrient medium and incubated in sealed bottles for up tp 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.
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 *Candida albicans*, *Aspergillus niger*, *Cladosporium sphaerospermum* or *Trichophyton mentagrophytes* had been added. The plates are then incubated at 28 °C either 2 days (*C albicans*) or 7 days (*A niger*, *C sphaerospermum* and *T mentagrophytes*). 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

Table 3d: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ISO/CD 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 *Staph aureus* or *K pneumoniae* 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 $ 1 order of magnitude during the incubation period.	Cell suspension intimate contact test.
ISO/CD 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 *Staph aureus* and *K pneumoniae* 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 ATM 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 $ 1 order of magnitude during the incubation period or by a measure of the variability of the data obtained.	Cell suspension intimate contact test.

Table 3e: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Textiles (fabric, yarn or pile / wadding)

Reference	Title	Description	Major Principle
ISO/CD 20743	Textiles - Determination of antibacterial activity of antibacterial finished products: Printing method	Replicate (6) samples of test material are either *Staph aureus* and *K pneumoniae* 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 ATM 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.

unsuitable for 'quantifying' the effect of the antimicrobial effects of treated textiles there are some disciplines in which they may provide data which is more relevant to the effect claimed than that delivered by a fully quantitative technique. For example, data has been presented to at least one international conference (Ref 10) on the efficacy of an antimicrobial agent incorporated into the lint of medical wound dressings. The demonstration of the effect in the presence of a suppurating wound was made by placing the dressing on an agar plate inoculated with a target species. After incubation, a zone of inhibition was present. The test dressing was then removed and placed onto a fresh inoculated plate and incubated. The cycle was repeated until no inhibition of growth was observed. The method appeared to provide useful support of the claim that the use of the dressing could inhibit the growth of bacteria in both the dressing and probably within the surface layers of the wound. It was claimed that the need to change dressings was minimised resulting in improved healing. The demonstration was supported by at least limited clinical data from trials using the dressings.

From Table 3 it can be seen that there are at least 4 existing techniques which provide quantitative data on the effect of treated textiles on bacteria. These present a second form of test and are typified by the method described in AATCC 100-1999 (see Figure 1) in which samples are inoculated with suspensions of bacteria and then incubated for a specified time before being examined for the size of the population present. The methods differ in the form of the suspension medium, number of replicates examined, test species and, to a certain extent, conditions for incubation. Methods AATCC-100 and JIS L 1902 appear to be the most commonly employed. The Swiss Standard SN195924 was based on AATCC 100 but was apparently modified to improve reproducibility and repeatability. This has resulted in a more complex procedure but suggests that issues of reproducibility are considered to exist. It is not known at this time how much information exists about such issues with the developers. These methods show clear potential as being suitable to determine both inherent bactericidal and bacteriostatic properties of textiles. Although primarily developed for examining effects against bacteria, they could be extended to the investigation of the impact on yeasts as well as fungal spores and mycelial fragments. The impact on other species of bacteria could readily be investigated. It is possible to envisage the method being extended to the examination of viral particles, algae and protozoa. In addition, such protocols could be combined with studies on ageing (*eg* the impact of washing cycles) to begin to satisfy at least some of the requirements of tests in Tier 2. Further studies on the impact of suspension media, reproducibility, repeatability and the scale and nature of the effect detectable would be required before a harmonised protocol could be produced. It is interesting to note that the International Bureau for the Standardisation of Man-Made Fibres (BISFA) recommend DIN prEN ISO 20645: 2001 - 12 for use with fibres equipped with leachable antibacterial finishes and ASTM E2149-01 (see Section 3.2 below) for fibres equipped with non-leaching antibacterial finishes.

A third form of test (ISO/CD 20743 - Printing method) places bacterial cells in direct contact with a textile without them being in aqueous suspension.

Figure 1: AATCC 100-1998 - Schematic Representation

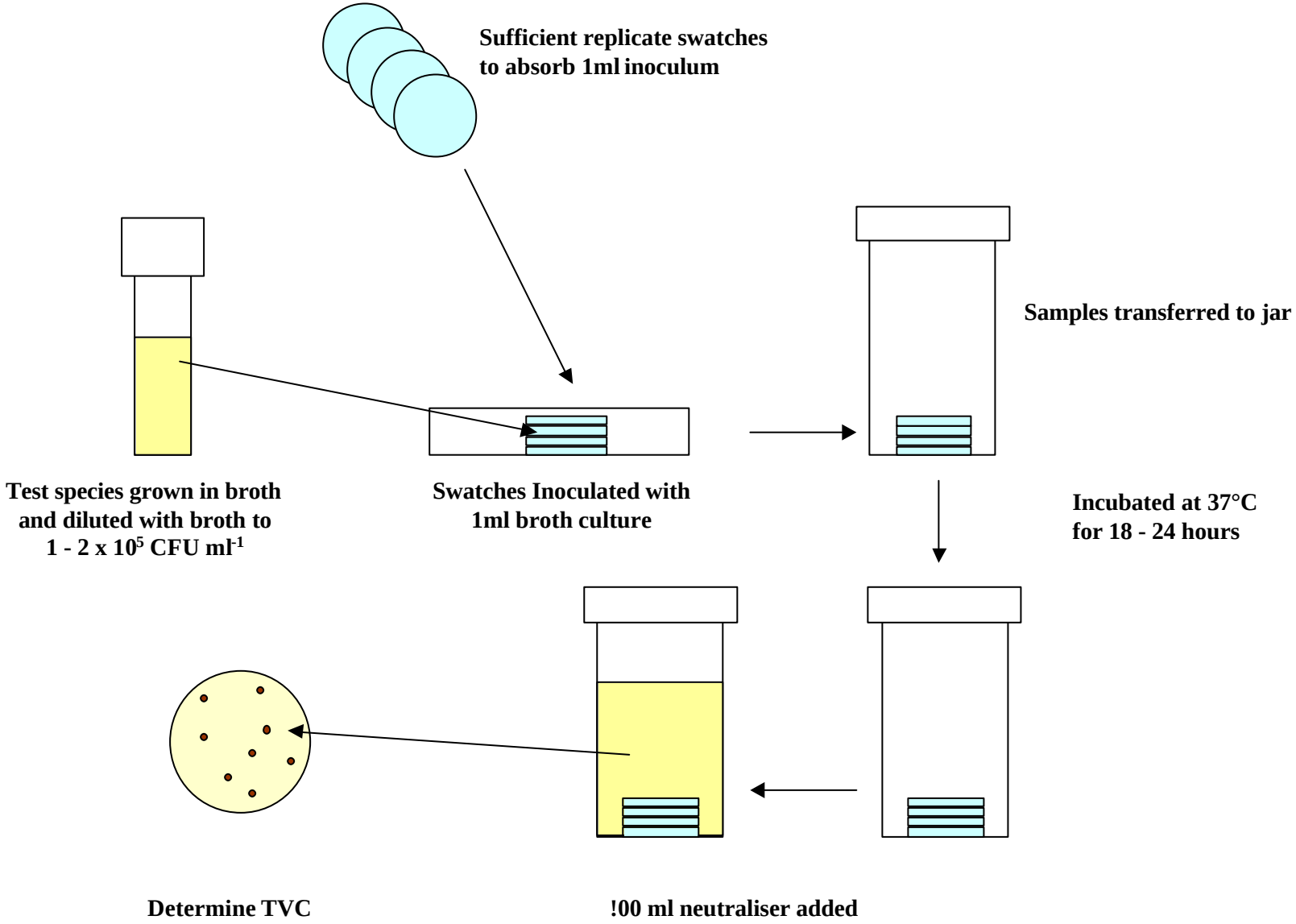


Table 4a: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Carpets

Reference	Title	Description	Major Principle
AATCC 174-1998	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 *Staph aureus* or *K pneumoniae*. 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
AATCC 174-1998	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 *Staph aureus* or *K pneumoniae* 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.

Table 4b: Existing Methods used to Examine the Antimicrobial Activity of Porous Surfaces: Carpets

Reference	Title	Description	Major Principle
AATCC 174-1998	Antimicrobial Activity Assessment of Carpets Quantitative Antifungal Activity	Petri dishes containing Sabouraud Dextrose Agar are inoculated with 1 ml of a spore suspension of *Aspergillus niger*. 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.
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 *E. coli* in nutrient broth is poured onto filter paper (7 cm diameter). The filter paper is then pressed for 1 min onto the surface of he 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 *E coli* by total viable count.	Cell suspension intimate contact test.
ASTM WK4757	Standard Test Method for the Assessment of Antimicrobial Activity In Carpets; Seeded-Agar Overlay Screen	Agar overlay method for assessing antibacterial and antifungal activity in carpets. FULL DETAILS NOT YET AVAILABLE	

It can also be seen from Table 3 that no fully quantitative methods exist for the examination of treated textiles on fungi. All of the protocols described are zone diffusion assays of one form or another. As with the antibacterial properties, these may be sufficient to substantiate certain claims (*eg* that a treated textile will not develop musty odours when stored under damp conditions or that certain fungi significant to infections on the human skin cannot germinate and grow on the textile). All of the methods described are performed in the presence of an agar supporting medium. However, it is possible that certain methods designed for the measurement of the potential for / prevention of biodeterioration could be employed dependent on the claim being made (*eg* prEN 14119 - see Table 6).

In addition to the truly microbiological methods describe above, at least 3 methods exist which describe the performance of woven textiles (Ref 11) and non-woven textiles (Refs 12 and 13) to penetration by bacteria under wet and dry conditions. They do not appear to have any direct relevance to the evaluation of treated textiles.

3.1.2 Carpeting

It can be seen from Table 4 above, that there are relatively few standards developed for the examination of the interaction of microorganisms with carpets. To a certain extent this appears to be because most of the studies on the biodeterioration of carpeting are either performed using methods designed for textiles in general or are performed on the components used to manufacture the carpet. However, the potential use of hygienic finishes in carpets / on the materials used to produce carpets have been the subject of work in the recent past, especially with regard to allergy to dust mites (Refs 14 and 15). This has had an implication both with regard to the impact of the microbiology of these floor coverings on mites (*ie* food sources *etc*) and in the ability to modify floor coverings such that they do not act as reservoirs of potential hazards to health (and offer them for use in clinical applications).

It can be seen from Table 4 that only 1 quantitative method for the examination of antibacterial finishes / treatments of carpets has been published (AATCC 174-1998). This standard also includes a method for the qualitative assessment of antibacterial and antifungal finishes (despite an indication that the latter is quantitative). A method similar to AATCC 174-1998 is being developed by WIRA however, it appears that the method is subject to a high level of variability and development is far from complete (Ref). ASTM are also working on a method for carpets. Full details have not yet been obtained but this appears to be a non-quantitative method (Ref 16).

In general, it would appear at first that the methods described in Section 3.1.1 could also be applied to carpets. The fact that AATCC have developed an additional standard for this substrate suggests that there are specific constraints which prevent this approach being adopted (probably size and bulk of the material). Similarly, it is possible that, at least for Tier 1 tests, the individual components of a carpet system (backing, fibres *etc*) could be examined separately. This needs to be explored further.

Table 5a: Existing Methods used to Examine the Antimicrobial Activity and Microbial Resistance of Porous Surfaces: Paper *etc*

Reference	Title	Description	Major Principle
DIN EN 1104	Paper and board intended to come into contact with foodstuffs Determination of transfer of antimicrobic 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 *Bacillus subtilis* or *Aspergillus niger* and incubated at 30°C for 7 days and at 25°C for 8 - 10 days respectively.	Zone Diffusion Assay.
ASTM D 2020-92	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 salts medium to determine if they support fungal growth.	Biodeterioration Test.
ASTM D 2020-92	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 / Biodegrdadation 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 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. 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 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. 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 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 Growth on specimens is assessed. Sucrose containing media is employed where true controls cannot be obtained.	Agar plate test

3.1.3 Paper, Board and other 'Natural' Materials

It can be seen from Table 5 above that no specific standards exist for the examination of paper and board as treated articles. ASTM D2020-92 examines the biodeterioration of paper and board and there are a number of methods available for the examination of the effect of preservatives of the 'wet blue' stage of leather. These are all relatively simple zone diffusion assays and do not address the requirements of treated articles. One method (DIN EN 1104) uses a zone diffusion assay to look for the presence of antimicrobial agents in paper destined for food contact applications. Again, this is unlikely to be suitable for the examination of treated articles / materials.

As with carpeting (Section 3.1.2), it is possible that methods developed for textiles might be applicable to paper and related products as might protocols for non-porous materials but further work would be required to confirm this. Certainly JIS Z 2801: 2000 (see Table 7a) has been adapted for the examination of paper coated with nanoparticulate titanium dioxide (Ref.17).

Table 6a: Existing Methods used to Examine the Resistance of Porous Surfaces to Biodeterioration: Textiles

Reference	Title	Description	Major Principle
prEN 14119	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 30-1998	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 *etc.*	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	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 micro-organisms. Preserved and unpreserved textiles are compared. Visual Assessment and measurement of tensile strength	Soil burial test
prEN ISO 11721-2	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 micro-organisms. 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)

Table 6b: Existing Methods used to Examine the Resistance of Porous Surfaces to Biodeterioration: Textiles

Reference	Title	Description	Major Principle
AS 1157.2 - 1999	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 *Aspergillus niger* 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 - 1999	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 *Chaetomium globosum* 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 - 1999	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 *Chaetomium globosum* 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).

Table 6c: Existing Methods used to Examine the Resistance of Porous Surfaces to Biodeterioration: Geotextiles

Reference	Title	Description	Major Principle
EN 12225	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 micro-organisms. Visual Assessment and measurement of tensile strength	Soil burial test

Table 6d: Methods under Development to Examine the Preservation of Wipes and Moist Non-Woven Textiles

Reference	Title	Description	Major Principle
EDANA Antibacterial Preservation V8	Recommended Test Method: Nonwovens - Antibacterial preservation	Test designed to determine the efficacy of preservation in non-woven textiles against bacterial contamination.	Agar plate test
Publication by A. Crémieux, S. Cupferman, C. Lens	Method for evaluation of the efficacy of antimicrobial preservatives in cosmetic wet wipes	Efficacy of preservative against fungi and bacteria is tested. A dry inoculum is placed into the original packaging among the wet wipes. The package is then re-sealed and assessed for growth over time.	Bacterial / fungal challenge test.

3.2 Existing Test Methodologies - Non-Porous Surfaces

It can be seen from Table 7 below, that a number of methods are available to examine the effect of non-porous treated articles / materials. Unlike with porous materials (see Section 2), few employ non-quantitative, zone diffusion assays although in practice these are used for the same illustrative purposes as for textiles. One (XP G 39-010) appears to employ the same methodology for both textiles and rigid polymeric surfaces. All of the methods concentrate on properties against bacterial populations. Two methods bring the microbial population into intimate contact with the test surface for a specified contact time. In both cases, the method attempts to overcome the problems of a hydrophobic interface between the material and the suspension of bacterial cells. In ASTM E2180, the bacterial cells are suspended in a lightly gelled agar 'biofilm' on the surface of the test substrate (see Figure 2). In JIS Z 2801 (and the ISO work item derived from it - Ref 18) a polyethylene film is used to maintain intimate contact (see Figure 3). Of the 2 methods, JIS Z 2801 appears to be the more widely used. Extensive work, including inter-laboratory ring tests was performed by the SIAA in Japan during its development (Ref 19). Further work has been performed in association with the International Biodeterioration Research Group (IBRG) and work between SIAA and IBRG is currently in progress in support of the ISO work item. This includes:

a Evaluation of within and between laboratory variability,
b Identification of a fully defined cell suspension medium,
c Validation of neutralisers and surface pre-treatments,
d Validity of the method for a range of biocide technologies.

Additional work not in support of the ISO work item includes the impact of temperature and time of incubation and the relationship with test species. This work is aimed more at expanding the use of the basic protocol into methods applicable to Tier 2 and beyond.

In addition to the tests described above that place a cell suspension in intimate contact with the surface of a test material, ASTM E2149-01 places pieces of a treated article into a cell suspension and measures the impact on the population following agitation in that suspension for a specified time. The method claims to be suitable for a range of materials including powders, textiles and rigid polymers. The method claims to simulate the dynamic contact between a treated material and a bacterial population and to be especially suited to non-mobile active ingredients. However, the method appears to be more suited to soluble active ingredients and is commonly employed by manufacturers of antibacterial plastic masterbatch materials as a quality assurance tool. Further information is probably required to assess the validity of the claims for this testing technology.

As with textiles, most of the quantitative testing methodologies described address the relationship between the treated material and a bacterial population. Again, some of these methods may well prove applicable to yeasts, fungal spores and mycelial fragments as well as algae, protozoa and

Figure 2: ASTM E2180 - Schematic Representation

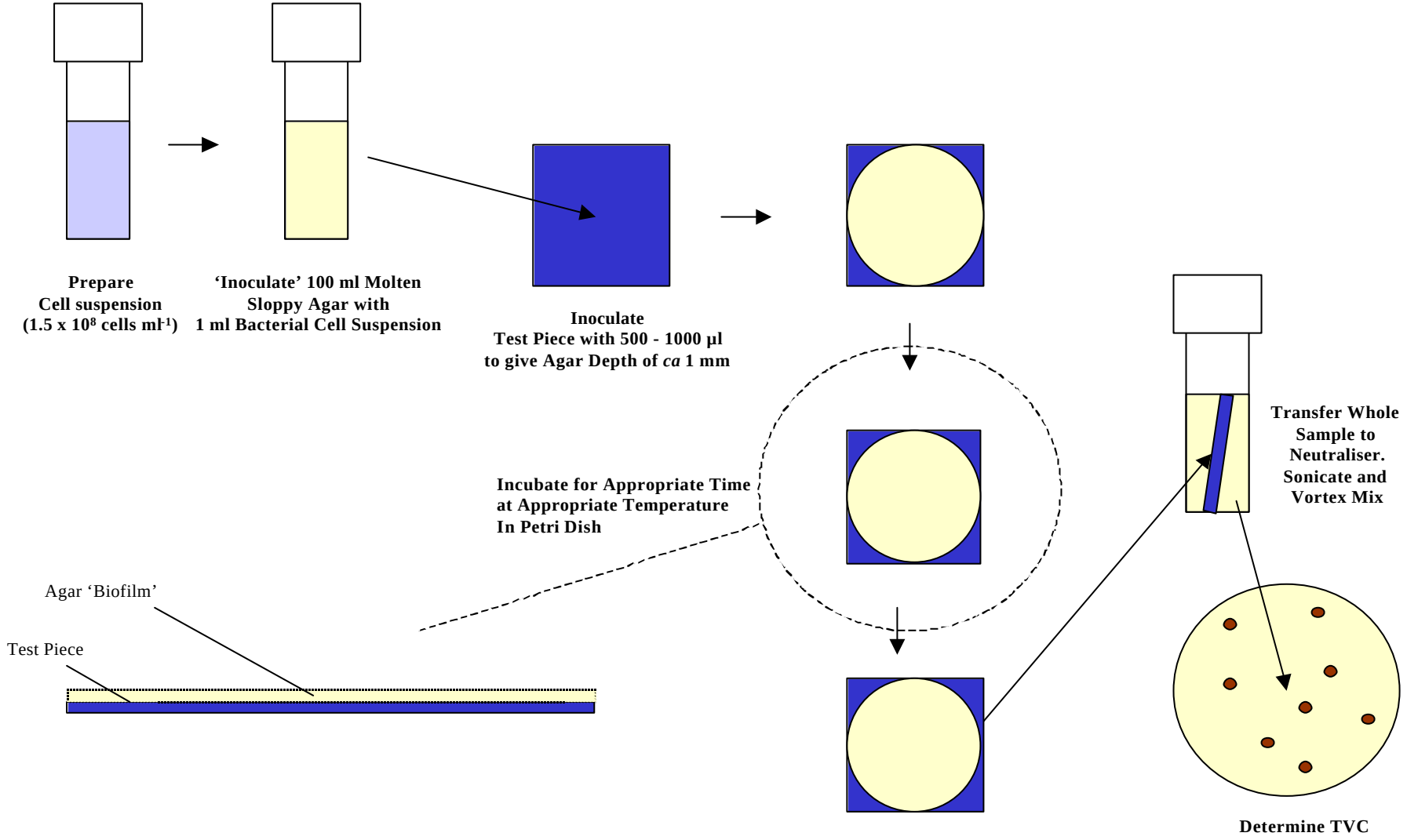


Figure 3: JIS Z 2801 - Schematic Representation

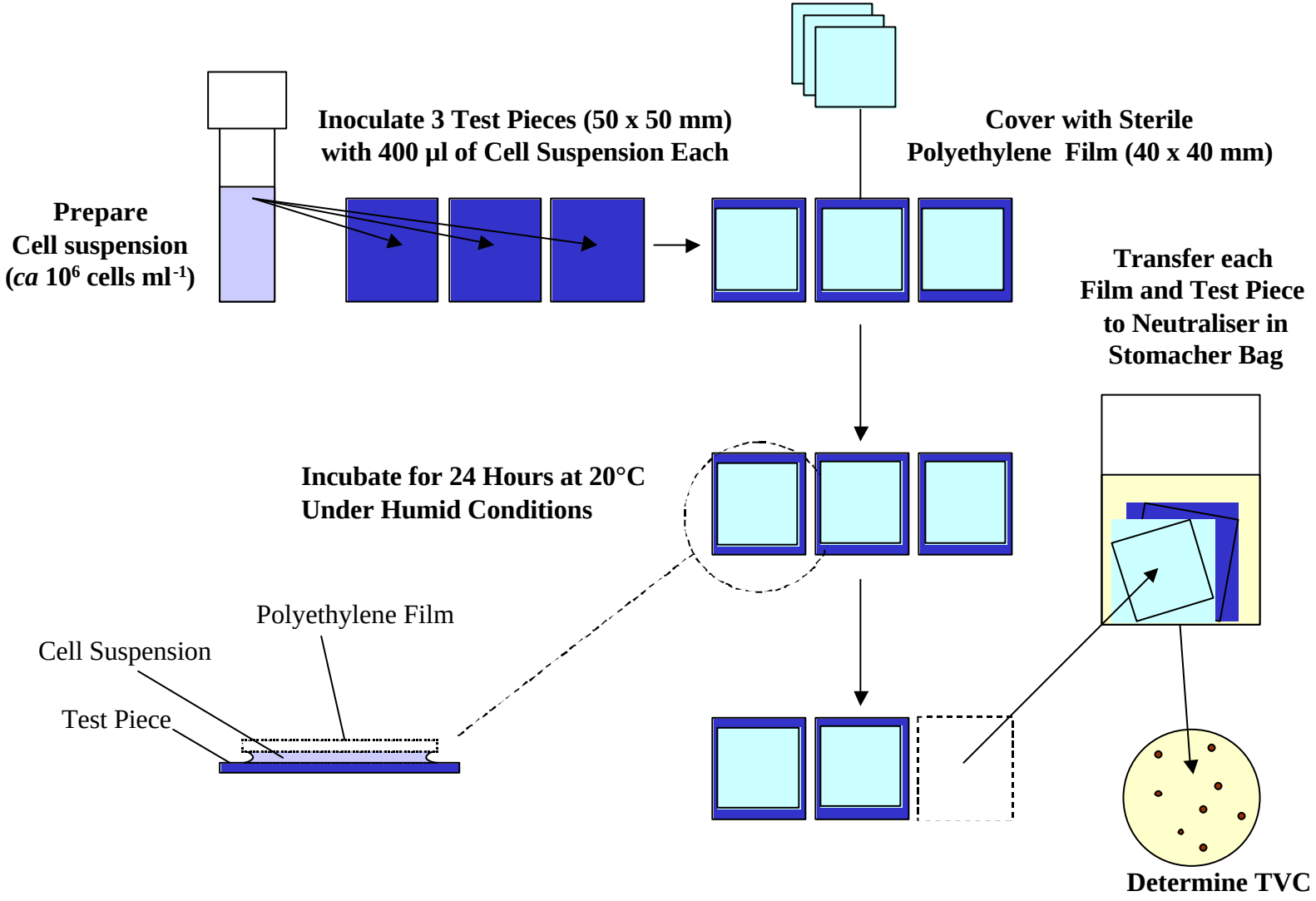


Table 7a: Existing Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
JIS Z 2801: 2000	Antimicrobial products - Test for antibacterial activity and efficacy	The surface of replicate sample (3 for each treatment and 6 for the blank reference material - usually 50 mm x 50 mm) are inoculated with a suspension of either *E coli* or *Staph aureus* 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 Log_{10} of the population on the treated sample and that on the control surface is > 2.	Cell suspension intimate contact test.
ISO / NP 22196	Plastics - Measurement of antibacterial activity on plastics surfaces.	This is the current New Work Proposal at ISO created from JIS Z 2801 by the SIAA of Japan. Modification and validation is in progress in collaboration with the IBRG. Some changes are expected.	Cell suspension intimate contact test.
XP G 39-010	Propriétés des étoffes - Étoffes et surfaces polymériques à propriétés antibactériennes - Caractérisation et mesure de l'activité antibactérienne	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 *Staph aureus* and *K pneumoniae* 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.

Table 7b: Existing Methods used to Examine the Antimicrobial Activity of Non-Porous Surfaces

Reference	Title	Description	Major Principle
ASTM E2180-01	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 *Staph aureus* or *K pneummoniae* suspended in molten semi-solid isotonic saline / agar. This attempts to form an 'artificial biofilm' which hold s the suspension in intimate contact with the test surface of inherentky 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 total viable count. Any effect is recorded using percent 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.	Immobilised cell suspension intimate contact test.
ASTM E2149-01	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 *Klebsiella pneumoniae* 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 mater 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.

Table 7a: Existing Methods used to Examine the Resistance of non-Porous Surfaces to Biodeterioration: Plastics

Reference	Title	Description	Major Principle
ASTM D 5338 - 92	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 - 99	Standard test method for evaluating the performance of antimicrobials in or on polymeric solids against staining by *Streptoverticillium reticulum* (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 - 76	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 - 96	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 visually assessed.	Agar plate test
ASTM G 29 - 96	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	Packaging - Determination of the ultimate aerobic biodegradability of packaging materials in an aequeous 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	Packaging - Determination of the ultimate aerobic biodegradability of packaging materials in an aequeous 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	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)

Table 7b: Existing Methods used to Examine the Resistance of non-Porous Surfaces 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	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 overlayed 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 *Chaetomium globosum* 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

Table 7c: Existing Methods used to Examine the Resistance of non-Porous Surfaces 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

Table 8a: Existing 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 ± 2°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 which may obviate this step. The method is validated by the need for fungal growth / germination of spores to be observed on a standard coatings 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 percent 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
D3273-00	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 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.	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 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.	Biodeterioration Test
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

Table 8b: Existing 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.
CEN Fungi	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
CEN Algae	Paints and varnishes – Laboratory method for testing the efficacy of film preservatives in a coating against alage	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 BPD. It is not intended to assess the performance of surface coatings.	Zone Diffusion Assay

Table 8c: Existing 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 *A niger* and *Penicillium funiculosum* . 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 *Scenedesmus vacuolaris and Stichococcus bacillaris* . 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
TESHSA NSI method	A non-suspended inoculum method for determining the antibacterial activity of coated surfaces.	A bacterial inoculum (either *E coli* or *Staph aureus* / *epidermidis*) is grown on the surface of solid media in a standardised manner and then used for the transfer of cells to replicate pairs of blank and treated surfaces. The size of the viable population on one of each of the replicate pairs of surfaces are analysed for the size of the viable bacterial population before incubation and the second is analysed after incubation at 24 hours at 20°C under humid conditions. A neutraliser is employed during recovery of the test population.	'Dry' inoculum intimate contact test.

viruses (see Section 3.3 below). At present many investigators employ methods such as ISO 846 and ISO 16869 to examine fungicidal and fungistatic effects. As discussed above, in some cases this may prove appropriate to the claim being made.

3.3 Surface Coatings

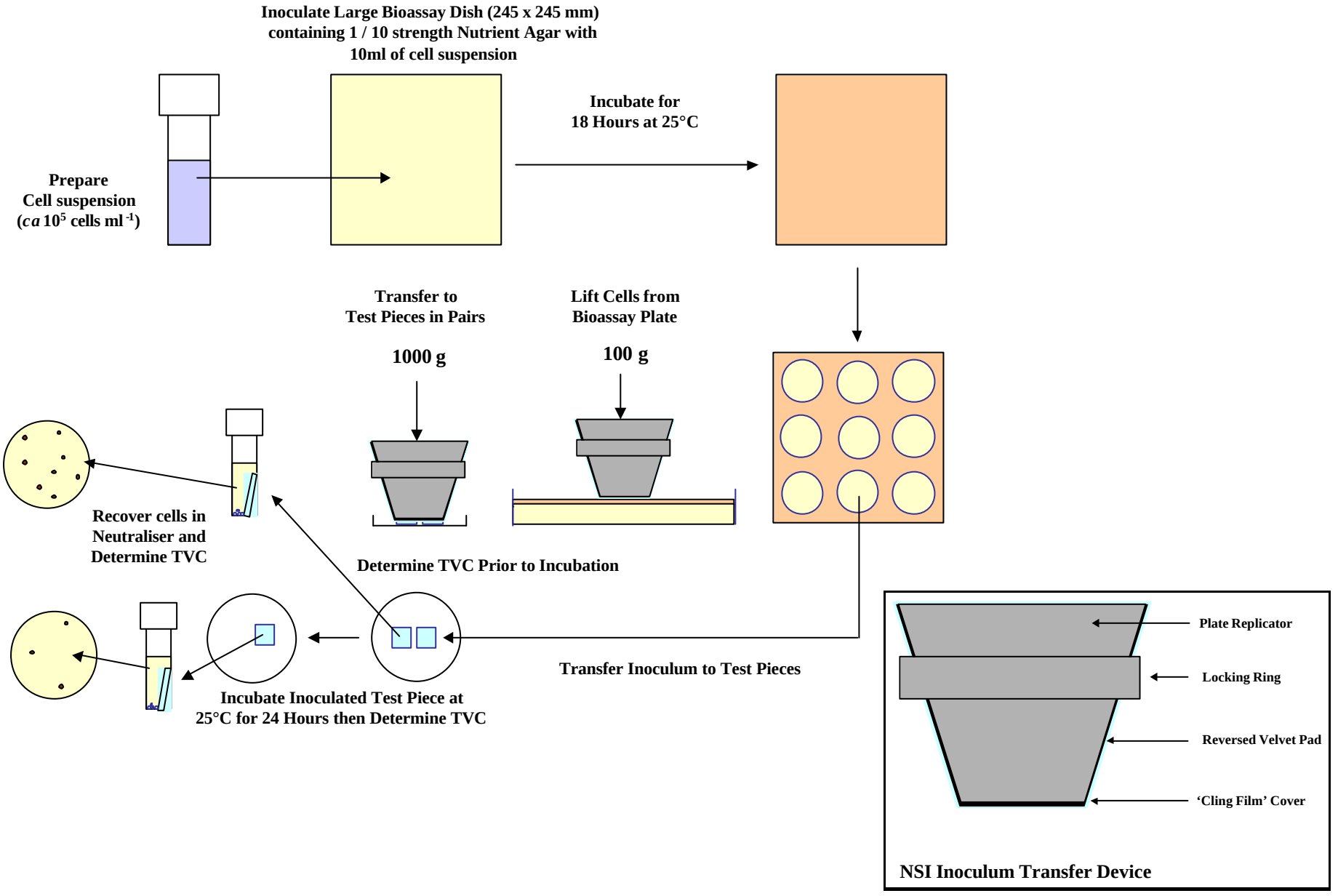
It can be seen from Table 8a and 8b above that all of the true published standards on coatings apply to the prevention of deterioration / defacement of the coating itself by either fungi or algae. As discussed with regard to porous and non-porous surfaces, in some cases these methodologies may be appropriate to a claim for a coating as a finish to a treated article. Indeed work has been reported in which JIS Z 2801: 2000 has been modified to accommodate bactericidal effects of wall paints (Ref 20) and it is commonly employed for assessing the performance of powder coatings intended to impart antibacterial finishes. Similarly, it has been modified to examine coatings which are intended to exhibit antiviral effects (Ref 21) utilising bacteriophage surrogates of infectious agents. Work has also been published which examines the interaction of bacteria with polymeric coatings over time by spraying them onto surfaces both with and without the presence of soiling agents and holding them under differing environmental conditions. Direct vital staining and epifluorescent microscopy is employed to measure the effects (Ref 22). It can also be seen from Table 8b that a protocol has been partially developed to examine the impact of coatings with claimed antibacterial surface properties on bacterial populations when presented in a non-suspended manner. In some ways this approach is similar to that described for textiles in ISO/CD 20743. The technique is illustrated in Figure 4 below.

4 Harmonisation of Testing Methodologies

4.1 Impact of Claims and Benefits on Choice of Methodology

During the initial stages of this project a questionnaire was sent to the appropriate regulatory authority of each member state of the OECD. The aim was to explore which types of treated article / treated material were authorised / regulated and whether any standard test protocols existed that were employed to generate data to support claims made. The responses to this questionnaire are shown in Appendix A. It can be seen that the scope / definition of treated articles / treated materials is, in general, consistent with that discussed in Section 1 of this report. Despite this, the terminology remains unclear and not well defined. For example, the term ‘treated article’ is employed in ASTM E 1428-99 to describe a polymeric material equipped with a biocide intended to prevent staining by actinomycetes and other microorganisms. Similarly, within the guidance documents for the implementation of the European Union Biocidal Products Directive (98/8/EC), the terms treated article / treated material are used extensively. In this case a distinction is drawn between an internal, preservative effect and an external biocidal effect with

Figure 4: Determining the Antibacterial Activity of Coated Surfaces using a Non-Suspended Inoculum



the intention to decide whether such a treated article / material requires authorisation as a biocidal product or not (Ref 23). The US EPA Antimicrobials Division also use the terms to reflect an effect external to the substrate but resulting from the substrate as discussed in Section 1 above (Ref. 24).

Data from the questionnaire (Appendix A) also demonstrate that many materials exist that appear to fall within the scope of treated articles / treated materials. Within these, a wide range of effects are claimed. The methods developed as standards and in use within industry reflect some of this diversity and make it difficult to identify a universal method suited to all. However, key to all of the materials is the concept of claim and benefit. This is discussed in some detail in Section 1 but it can be expanded to provide further reference for deciding what form harmonised methods should take and what they should encompass.

During the OECD Biocides Workshop (Ref 4) some discussion centred on the type of effect that might be measured as well as what form and how large that effect should be to be declared valid. Much of this will depend on the nature of the claim made / intended and the size and nature of the effect required to provide a benefit. The method used to substantiate the claim and support the product should be capable of demonstrating the effect in a scientifically valid and statistically sound manner.

As mentioned before, the terminology applied in this area is sometimes unclear. A possible solution to the problem of definitions might be resolved by separating the term treated material and treated article. A treated material could simply include any material which has been fortified / modified in some manner such that it exhibits a microbiological effect. This effect might be preservation (an internal effect), the transfer of a disinfectant to another material (delivery mechanism, *eg* disinfectant wipes) or affect the status of microorganisms that come into contact with it for some hygienic process (an external effect). A range of methods (both existing and new) might be used to demonstrate these effects. These treated materials might then be employed to manufacture an item. This item itself might now be intended to elicit a microbiological effect external to it and be deemed as a result to be a treated article. For example, a fungicide might be employed to protect PVC from fungal attack, preventing both discolouration and loss of flexibility. A range of methodologies are available (*eg* ISO846) which can be used to demonstrate the material is protected from deterioration as a result of microbiological activity in service. The use of the fungicide is clearly intended to protect (preserve) the PVC in service. No external effect is claimed and this remains a treated material (with no external effect). In another case a powder coating might be formulated with the addition of an antimicrobial agent creating a treated material. Coated substrates can be shown to demonstrate antimicrobial activity to a range of bacteria that pose no threat to the coating itself including some which are considered to have clinical significance (*eg* MRSA). Data is produced which demonstrates that powder coated surfaces can reduce bacterial populations to below limits of detection within 24 hours at 37°C in a intimate contact test such as JIS Z 2801. Based on the above, we now appear to be dealing with a treated article (the powder coating produces an

external effect and transforms the substrate into a treated article). Basic efficacy has been demonstrated and we will assume that the data is both scientifically and statistically valid. The coating is used to produce door handles intended for use in clinical units. The intention of the product is to improve hygiene in clinical units and reduce the risk of cross infection with agents such as MRSA. However, one must now consider the claim in respect to the benefit implied. Can data be produced which can demonstrate that the powder coated door handle can operate with sufficient speed to prevent an infectious agent on the hands of one person being transferred to a second if they should use the door handle within minutes / seconds of each other? Is there a significant benefit achieved through the use of this powder coating? Obviously this implies that every potential application may need to be considered with respect to claim and benefit (this would also have taken place during the initial market surveys for the product in any case) and some specific data may be required. However, in many cases framework formulation data may be sufficient (*eg* data from powder coated test panels would probably be sufficient to support the use of the coating on a range of products provided it supported the claim made and the benefit implied).

In the scenario above, claim (or in some cases intent) and benefit become the key components in the decision about the status of a product being a treated article. Methods must be available to demonstrate basic efficacy and these methods must be scientifically valid and be capable of resolving the level of effect seen in a statistically valid manner. The data produced must be shown to support the claimed benefit of using the treated article in a specific application. This may require either additional data or a soundly reasoned case.

4.2 Harmonised Test Methods for Tier 1 Tests

4.2.1 Porous / Absorbent Materials

It can be seen from Section 3 above that a number of methods exist which take common routes to the quantitative determination of activity of treated articles towards bacteria. In textiles, this is typified by AATCC 100 (see Figure 1 above). The method utilises a model Gram Positive bacterium (*Staphylococcus aureus*) and a model Gram Negative bacterium (*Klebsiella pneumoniae*), utilises both treated control samples, allows replication (and includes some measure of homogeneity in the test material) and incorporates a neutraliser during the recovery of the populations from the textile. Variations on this approach (*eg* SN 195924) utilise a different model Gram Negative species (*Escherichia coli*) and increase replication. Some provision is made for alternative contact times and temperatures and other species can be accommodated. This would probably include yeasts, fungal spores, hyphal fragments and virus particles. At present, little is known about the level of variability inherent in the method (both intra and inter-laboratory) however, it is understood that investigations into this were undertaken during the development of SN 195924 (Ref 25). The basic requirements for harmonisation appear to be fulfilled by this form of test for textiles and many other porous materials although data is required

to provide a measure of the size and nature of any effect seen using it. The method should prove adaptable to allow the investigation of both biostatic and biocidal functionality. Introduction of soiling agents and other interfering substances would also be possible. The effects of pre-treatments (*eg* laundering, UV irradiation *etc*) on antimicrobial efficacy could be investigated with relative ease. As with many of the tests, this type of protocol presents the inoculum to the substrate as a cell suspension. No attempt is made to simulate dry deposition of microorganisms and constant, virtually saturation humidity is maintained during the incubation period. The test is very much a basic efficacy test and matches well the requirement for a Tier 1 test in which potential activity is investigated (*ie* proof of principle) however, it is possible that data derived from it could be used to validate certain specific claims directly and modifications (such as alternative humidity and temperature regimes) could allow other factors to be studied.

Although the AATCC 100 model could be adapted to examine fungicidal properties, it would probably be less well suited to investigating fungistatic properties except with certain yeast species. Growth of filamentous fungi may be difficult to quantify using the method and conditions (*ie* textiles saturated with growth media) may not be ideal for the growth of many species. A humid chamber type test (*eg* BS 2011 : Part 2.1J) may prove more suited with some form of rating system being used to measure growth over time.

4.2.2 Non-Porous Materials and Surface Coatings

It can be seen from Section 3 that although a wide range of tests exist to assess the susceptibility, biodegradability and protection of non-porous materials and coatings, there are relatively few that focus on them as treated articles as defined above. Two approaches are taken to provide a quantitative measure of the activity of non-porous treated articles on bacteria. In ASTM E2149-01 the material is placed into a suspension of bacterial cells in a buffered solution. The size of the bacterial population is measured following incubation with agitation for a specified contact time. It is claimed that the 'dynamic' nature of the test ensures contact between bacterial cells and the test substrate and that it is ideal for materials equipped with non-mobile active ingredients. The converse however, appears to the case and the second approach appears much better suited to these materials. In JIS Z 2801 and ASTM E2810, a bacterial population is held in intimate contact with the surface of a treated article for a specified time and at a specified temperature. In JIS Z 2801 a sterile polyethylene film is used to maintain this contact and in ASTM E2810 a semi-solid agar is employed (see Figures 2 and 3). The former approach introduces less organic matter and can achieve closer contact between the population and the surface and appears well suited to the measurement of potential activity as required for a Tier 1 test. It is currently a new work item within ISO and is being developed further through collaboration with the International Biodeterioration research Group (IBRG). Extensive data is already available to support the method (Ref 26) and further validation and statistical evaluation is in progress through international ring tests organised by IBRG. This method is well recognised throughout industry and little resistance to adoption as a basic efficacy test would be expected.

The method has been adapted to allow the investigation of coated surfaces (Ref 20) and to examine activity against viruses (Ref 21). As with the textile methods discussed in Section 4.2.1 above, the method could also probably be adapted to examine fungicidal claims but fungistatic activity would probably be better examined using an alternative technique. Again, a humid chamber type test such as that described in BS 2011 : Part 2.1J might better suitable. The JIS Z 2801 type of protocol could also be used to examine claims beyond the scope of a Tier 1 test for materials exposed to constant humidity. Temperature and contact time variations would be relatively simple to integrate. As with porous materials, such a Tier 1 test would probably not be suited to investigate the activity of treated articles on microorganisms deposited under dry / semi-dry conditions (*eg* deposition of bacterial endospores from the air and from skin contact). It could probably be adopted to study the effect on microorganisms deposited in droplets / from splashes however, by the use of different levels of humidity during the incubation phase.

5 Recommendations

As discussed above, treated articles encompass a wide range of materials. The claims made for these materials are equally diverse. However, at the most basic level the materials can be divided into two broad categories depending whether the finished article is either porous (*eg* most textiles, paper) or non-porous (*eg* plastics, surface coatings). Similarly, the effects claimed essentially fall into two categories depending on whether the overall effect is intended to be either biocidal or biostatic. Unlike most disinfectant applications, the size of the effect will be related to the claim made for the article. An arbitrary measure of performance (*eg* reduction by 5 orders of magnitude following contact for 5 minutes) is not useful for treated articles. The purpose of the testing methodology is purely to provide a mechanism to show that any effect claimed can be demonstrated in a scientifically and statistically significant manner.

As described above and proposed during the workshop described in Reference 4, it is envisaged that the data used to support claims made for treated articles will be generated through the use of a hierarchy of test requirements starting with the demonstration of the basic properties claimed (Tier 1 and the purpose of this review). The usefulness and overall impact (whether positive or negative) of the claimed effect are not relevant when demonstrating the basic effect but will need to be considered and demonstrated using data specific to the final application. Thus for example, if a plastic material is claimed to be able to prevent the growth of bacteria on its surface, Tier 1 data would need to demonstrate this (*ie* no increase in the population of both model Gram Positive and Gram Negative bacteria should occur under defined conditions). If this plastic is intended to be used to manufacture the lining materials for refrigerators, the casings for domestic food processors and components used in the air conditioning system of cars further, more application specific data will be required in Tiers 2 and 3 (*eg* the demonstration of the effect against psychrophillic bacteria under conditions present in refrigeration systems, the relevance of the effect when no surface water is present *etc*). It is possible in some circumstances that the basic data will be generated by either the manufacturer of an additive or a system (*eg* plastic master

batch, powder coating system), whereas the more application specific data will be generated by the manufacturer of the finished article (possibly in collaboration with the supplier). This would enable decisions about relevance and impact to be assigned to end products / applications rather than to technology alone.

During the review of currently available test methodologies two basic approaches were identified that appear to be highly suited to form the basis for a harmonised approach.

Porous Materials

For porous materials the method typified by AATCC 100-1998 appears to demonstrate the basic requirements for a tier 1 test.

1 It describes a method which generates fully quantitative data.

2 Modification could accommodate both biocidal and biostatic effects

3 Modification could accommodate a wider range of microbial species (including certain fungi, algae and protozoa as well as a wide range of bacterial species and viruses).

4 Modification could allow a wide range of contact times and temperatures to be examined.

The method has been adapted to form the basis of other national standards and a variant is included in the current draft ISO standard (ISO/CD 20743). Data relating to the within test, between test and between laboratory variability will be required to enable decisions related to the size of effect that can be detected to be made. This data is known to be available for the Swiss national standard variant for bactericidal properties (this variant appears to form the basis for the draft ISO standard) but data from AATCC has not been made available. Data will be required for variants that look at biostatic properties and for variants that examine other types of microorganisms. This data would need to be generated through collaborative ring tests. Some modifications would be required to facilitate the validation of suitable neutralisation systems and pass / fail criteria would need to be omitted.

The basic protocol could be adapted to provide some of the data for the more specific requirements of Tiers 2 and 3 (*eg* the impact of temperature, soiling agents, laundry cycles *etc*). Again, further ring tests would probably be required to both design and validate these adaptations. During the review, it was noted that the method could be adapted for examining effects against a wide range of microbial species but that it was not considered suitable for assessing the impact of a porous treated article on the growth of filamentous fungi and algae (due to the difficulties in

producing a meaningful quantitative measure of growth). A number of standard methods exist which examine the susceptibility of porous materials to fungal growth and determine the efficacy of treatments intended to prevent fungal spoilage. Many of these could be employed to generate data relevant to demonstrating the effect of treated articles on fungal growth within Tier 1. Many of these methods use agar (either with or without a full range of nutrients) plates to provide the humid conditions required to achieve fungal growth. Others employ some form of humid chamber. It is likely that variants of the latter would be more suitable for generating data for at least some of the more specific requirements of Tiers 2 and 3, while the former could be used to demonstrate basic inhibition of fungal growth. Modifications of either these or methods described for non-porous materials (see below) could be used to study the impact on algal growth when required.

Non-Porous Materials

For non-porous materials the method typified by JIS Z 2801: 2000 appears to demonstrate the basic requirements for a tier 1 test.

1 It describes a method which generates fully quantitative data.

2 Modification could accommodate both biocidal and biostatic effects

3 Modification could accommodate a wider range of microbial species (including certain fungi, algae and protozoa as well as a wide range of bacterial species and viruses).

4 Modification could allow a wide range of contact times and temperatures to be examined.

The method has been adapted to form the basis of a draft ISO standard (ISO / NP 22196). Although ASTM E2180 utilises a similar basic approach (attempting to hold a suspension of bacterial cells in intimate contact with the surface of the treated article), the presence of the agar film has been demonstrated (unpublished data produced during an industry sponsored study at the PRA / IMSL, UK - see Tables 9 and 10) to have a negative impact on certain antimicrobial agents commonly used in treated articles (*eg* Ag^+ ions). For this reason it does not appear to be suited to the generation of tier 1 data for all treated article types.

Data relating to the within test, between test and between laboratory variability will be required to enable decisions related to the size of effect that can be detected to be made. This data is being produced in support of the draft ISO version of JIS Z 2801: 2000 and is available for JIS Z 2801 itself (Ref 19). Data will be required for variants that look at biostatic properties and for variants that examine other types of microorganism. This data would need to be generated

through collaborative ring tests. Some modifications would be required to facilitate the validation of suitable neutralisation systems and pass / fail criteria would need to be omitted (it is anticipated that this will take place during the review process of the draft ISO variant).

As with porous materials, the basic protocol could be adapted to provide some of the data for the more specific requirements of Tiers 2 and 3 (*eg* the impact of temperature, soiling agents, cleaning cycles *etc*). Again, further collaborative ring tests would probably be required to both design and validate these adaptations. During the review, it was noted that the method could be adapted for examining effects against a wide range of microbial species but that it was not considered suitable for assessing the impact of a porous treated article on the growth of filamentous fungi and algae

Table 9: Determination of Activity against *Staphylococcus aureus* using JIS Z 2801

Description	CFU cm^{-2}	
	Time 0	Time 24
No Antibacterial	5.8×10^3	1.1×10^4
Silver Based Antibacterial		< 12

Table 10: Determination of Activity against *Staphylococcus aureus* using ASTM E2180

Description	CFU cm^{-2}	
	Time 0	Time 24
No Antibacterial	1.2×10^5	3.4×10^6
Silver Based Antibacterial	1.2×10^5	2.4×10^6

(due to the difficulties in producing a meaningful quantitative measure of growth). A number of standard methods exist which examine the susceptibility of a wide range of non-porous materials to both fungal and algal growth and to determine the efficacy of treatments intended to prevent spoilage by such growth. These could be employed to generate data relevant to demonstrating the effect of treated articles on fungal / algal growth within Tier 1. Many of these methods use agar (either with or without a full range of nutrients) plates to provide the humid conditions required to achieve fungal / algal growth. Others employ some form of humid chamber. It is likely that variants of the latter would be more suitable for generating data for at least some of the more specific requirements of Tiers 2 and 3, while the former could be used to demonstrate basic inhibition of fungal / algal growth.

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Appendix A: Compilation of Survey Data for OECD Countries For Project on Harmonizing Efficacy Test Methods - Treated Articles

QUESTION	Does your country directly regulate articles/materials treated with biocides?	If no, are there plans to introduce such a process soon?	Do you regulate the biocides used to treat the materials?
COUNTRY			
Finland	There is no advance approval system for articles/materials treated with biocides	Yes, related to BPD.	In future, related to BPD.
Great Britain	No - With the exception of a few products under COPR (e.g. impregnated insecticide strips).	Yes such products will be regulated under the BPD. The issue of what is a treated article and what types of product fall under the scope of the BPD (98/8/EC) is covered (with examples) in the EU commission document "Guidance on treated material/articles and some other scope issues" which can be found with other useful documents on scope, definitions etc. on the EU Commission website at *http://europa.eu.int/comm/environment/biocides/index.htm*	Yes.
Netherlands	Yes, efficacy regulation for certain products, f.i. food contact materials, and in case of claims concerning disinfection capacity (eg. disinfecting wipes)		Yes (EC Biocidal Product Directive 98/8)
Italy	No.	No.	Yes.
Sweden	No.	No.	Yes.
USA (EPA)	Yes. However, materials with biocides that are incorporated into the material to protect or preserve the material are considered treated articles and are exempt from registration. For additional information, please see the following	N / A	Yes.

	http://www.epa.gov/opppmsd1/PR_Notices/pr2000-1.pdf Those treated materials which make public health related label claims are subject to registration.		
USA (California)	Yes, if the treated articles make biocidal claims, e.g. bacteristatic water filter and towelettes	N / A	Yes
Japan	No.	No.	No.
France	Some wipes impregnated with "antiseptics" may be considered as medical devices or as drugs…	I have no precise information but that is linked to the "Biocide directive" application.	I have no precise information but that is linked to the "Biocide directive" application.
Germany	No.	There will be a regulation by the European Union	Yes.
Australia	These are not regulated by the TGA. Such products fall under the Australian Trade Practices Act and Consumer Affairs, but as far as is known, the efficacy of such products is not regulated other than in relation to advertising claims.	Unknown	If the biocide used to treat materials comes under TGA's legislation as described above, the biocide is assessed according to TGA's system as described above. If the chemical is a new chemical entity, it is also assessed by NICNAS (National Industrial Chemicals Notification and Assessment Scheme). If the biocide used is not covered by TGA's legislation (agricultural disinfectants, water disinfectants etc), it is assessed by other agencies, eg. APVMA (Australian Pesticides and Veterinary Medicines Authority).
Czech Republic	Yes, if the treated articles make antimicrobial (biocidal) claims.		Related to BPD (better to say according its Czech version)

QUESTION	**If yes, what conditions (if any) are placed on the biocides directly relating to their use in materials? Example: the biocide registration must be evaluated for the safety of such a use.**	**Do you have policies, standards or guidelines for the generation of microbicidal efficacy data in the treated article/material?**	**If you have any standard test methods for specific articles/materials, please provide electronic or hard copies of the methods or a reference to where copies could be obtained by the task force.**
COUNTRY			
Finland		No	Not available.
Great Britain	If the treated article falls under the scope of the BPD then, as with all other biocidal product types under BPD the active substance (& product) will be assessed for its safety and its efficacy. UK/COPR does not consider use of treated materials and disposal issues, BPD does.	There are very few standards currently available – what is available would have been highlighted at the OECD Biocides Workshop in 2002 – see answer to Q14,	The most substantive list we are aware of was prepared at the OECD Biocides Efficacy workshop. A copy of the workshop report together with the list of methods for treated materials is available on the OECD Biocides website: *http://webdomino1.oecd.org/comnet/env/Workshops.nsf/Documents/bio-efficacy/$File/index.htm* *Username: Wood Account; Password WAccount.*
Netherlands	Claims should be substantiated, no specific requirements, except for food contact materials (Note for Guidance, Chapter III, SCF-WG Explanatory guidance www.europa.eu.int/comm/food/fs/sfp/food_contact/index_en.html)	Yes, qualitative tests: 1)Kirby Bauer Agar diffusion test;2)TNO Seedlayer method; and quantitative tests: 1) Japanese standard JIS Z 2801 (2000) Antimicrobial products- Test for antimicrobial activity and efficacy; 2) IBRG Method to determine antimicrobial activity of plastics.	If desired copies can be provided
Italy	Biocide registration implies also an evaluation for safety in use	No.	
Sweden	Risk assessments on the use of the biocidal product on the treated article and on the use of the treated article in turn, would be used to assess the	No.	No.

	acceptability of such uses. Any necessary conditions can be placed on a product authorisation – if an authorisation is granted.		
USA (EPA)	The biocide incorporated into the treated material would be evaluated for safety (always) and efficacy (if public health claims are made on the article.)	EPA does not have methods or performance standards for evaluating the public health efficacy claims of treated materials.	N / A
USA (California)	The biocides need to meet the general requirement for registration as a pesticide in California. In addition, we require specific efficacy data derived from in-use or simulated in-use condition on the materials to be treated over a period of time as defined on the product label. Untreated control samples are normally required.	We have no written policy, standards or guidelines specific for treated articles. The unwritten policy is that efficacy data need to be generated under use or simulated use condition to substantiate the treated articles' claims.	For bacteriostatic water filters, we accept the NSF methods, (National Sanitation Foundation, 3475 Plymouth Road, P. O. Box 1468, Ann Arbor, Michigan 48106, U.S. A.) For towelettes, we use the U.S. EPA method.
Japan	N / A	"Guidelines for Antimicrobial Products" was developed by the Ministry of Economy, Trade and Industry in October 2002 and available from the following Web Site. http://www.meti.go.jp/english/information/downloadfiles/cAntimicrobiale.pdf As a testing standard, Japanese Industrial Standard (JIS) Z 2801 "Antimicrobial products-Test for antimicrobial activity and efficacy" was established in December 2000.	English version of the JIS can be obtained from the Japanese Standards Association. http://www.webstore.jsa.or.jp/webstore/Top/indexEn.jsp
France		The policies and guidelines I have are those of our lab. They are aimed at testing a defined antimicrobial activity on a defined material	Publication in preparation..
Germany	Biocides need an admission by the Federal Institute for Occupational	Yes.	Yes, your will get them by Dr. Gebel

	Safety and Health according to the Biocide Act		
Australia	Unknown - NICNAS evaluate new chemical entities for safety.	Unknown	Unknown
Czech Republic	The biocide used must be assessed for its efficacy, safety (inc. non-irritant properties on skin)	A semi-quantitative carrier test is used	The protocol (method no 3) has been already sent to the OECD secretariat.

QUESTION	**If the requested material is not available in English or French, can a translation in either one of the two languages be provided?**	**Please provide a list of the kinds of treated matrices in your country. Examples: clothing, kitchen utensils, shower curtains, coatings.**	**Could you describe the range of claims being made in conjunction with treated articles/materials in your country? Example: antibacterial, self-sanitizing**
COUNTRY			
Finland		Sports clothing, socks, insoles, tents, kitchen utensils, tights	Antibacterial, antimicrobial.
Great Britain	The most substantive list we are aware of was prepared at the OECD Biocides Efficacy workshop. A copy of the workshop report together with the list of methods for treated materials is available on the OECD Biocides website: *http://webdomino1.oecd.org/comnet/env/Workshops.nsf/Documents/bio-efficacy/$File/index.htm* *Username: Wood Account; Password WAccount.*	Although not currently regulated under national legislation, there are a wide number of treated materials available on both the UK Examples include: Textiles, clothing, work wear, gloves, paint coatings, food preparation utensils (e.g. chopping boards), food contact surfaces, food packaging, flooring materials, walling materials, etc.	Examples (not exhaustive) that have been seen on the UK market include: Socks…….”improved freshness” Socks….”helps keep your feet fresh and odour-free” Kitchen sponge…”Helps prevent the spread of germs” Sticking plaster…”kills germs, helps prevent infection” Floor cloth…”built-in protection against bacteria” Sports socks….”inhibits the growth of odour-causing bacteria” Lavatory brush….”Helps prevent the spread of bacteria on the body of the brush” Anti-bacterial fabric….”Effective control and growth prevention of a wide range of gram positive and gram negative bacteria, fungi, algae and yeasts” Hygienic paint coating…”prevent the spread of germs, reduce the risk of infection and protect (us) from harmful bacteria” Antimicrobial flooring….”effective antibacterial and antifungal protection,

			ensuring the floor remains free of bacteria between cleaning cycles" Hygienic steel coating…"neutralises the ability of bacteria to function, grow and reproduce" Multi-surface coating…."kills in contact within 4 hours" Tights…"combats the growth of the yeast and fungus that cause thrush and athlete's foot" *N.B. some of these claims are clearly aimed at biostasis, others describe a disinfection action.* Currently none of these products are subject to any regulation (with regards to their efficacy). Some will fall under the scope of the BPD. For those that do, then claims will have to be substantiated through the provision of robust and scientifically sound, quantitative efficacy data.
Netherlands		Conveyor belt and other food contact materials, including kitchen utensils; active packaging materials; ceiling and wall panels; flooring materials; cleansing wipes; shower curtains, clothing; footwear; incontinence diapers (odour control).	-Antimicrobial; -Antibacterial; -Antifungal; -Hygienic (microbial growth inhibition); -Odour control.
Italy			
Sweden			
USA (EPA)	N / A	Exempted treated articles: sponges, cutting boards, cloths, paints	Please see the following http://www.epa.gov/opppmsd1/PR_N

			otices/pr2000-1.pdf
USA (California)	N / A	Bacteriostatic water filter and towelettes. (So far, most of the treated articles with health related claims are either unregistered products doing illegal sales, or ideas in the research and development stage. There are also products that had deleted their health related claims when notified by authority the need for registration as a pesticides. Examples for these types of matrices or articles are: lunch box, sponges, pencils, chopping board, clothing, kitty litter, etc.)	Antibacterial, bacteriostatic, disinfecting, sanitizing, and self-sanitizing.
Japan	N / A	Plastics (chopping board, kitchen knife handle, toothbrush, wrapping film, toilet seat, comb, refrigerator, mobile phone, rice cooker, steering wheel, shift knob, door knob, pencil, floppy disk case, desk mat), Textile (socks, insole, towel, carpet, pillow, wall paper), Ceramics (dish, tile, toilet, porcelain enamel), Metal (stainless steel)	Definition of "antimicrobial" is stipulated in the above-mentioned Guidelines for Antimicrobial Products as follows. "Antimicrobial" in "antimicrobial products" shall be defined as "inhibiting the growth of bacteria on the surface of products."
France		Textiles (for multiple uses).; wipes; plastics (for multiple uses); paper (for packing).	“antibacterial” is the most commonly used claim (probably because this word doesn’t correspond to an activity required by our standards….
Germany		Clothing, tile, laminate floor, door handle.	There are no official terms.
Australia		Clothing, kitchen utensils (chopping boards), plastic garbage bins and bags, antibacterial paints and kitchen wipes that kill organisms actually inside the wipe (rather than on the surface) are all on the market in Australia.	The most common would be antibacterial claims. There is no definition for this in TGO 54. There is a voluntary code of practice for antibacterial cleaners (for bathroom purposes) which defines antibacterial as a 3-log reduction in test organism (these products are excluded from TGA's regulation).

Czech Republic		socks, ceramic tiles	antibacterial, antimicrobial (for e.g. antifungal effect)