BSI Standards Publication

Quantitative surface test for the evaluation of residual antimicrobial (bactericidal and/or yeasticidal) efficacy of liquid chemical disinfectants on hard non-porous surfaces – Test method

ISBN 978 0 580 84408 9

ICS 71.100.35

Publication history

First published November 2014

Amendments issued since publication

Date	Text affected

Summary of pages
This document comprises a front cover, an inside front cover, pages i to iv, pages 1 to 32, an inside back cover and a back cover.

This page deliberately left blank

This PAS was sponsored by Byotrol Technology Limited. Its development was facilitated by BSI Standards Limited and it was published under licence from The British Standards Institution. It came into effect on 30 November 2014.

Acknowledgement is given to the following organizations that were involved in the development of this PAS as members of the steering group:

- BluTest Laboratories
- Campden BRI
- Health and Safety Executive (HSE)
- Hospital Infection Research Laboratory, Queen Elizabeth Hospital Birmingham
- International Biodeterioration and Biodegradation Society (IBBS)
- JSC International
- Klarus Consulting
- Organic Materials Innovation Centre (OMIC), The University of Manchester

Acknowledgement is also given to the members of a wider review panel who were consulted in the development of this PAS.

This PAS is not to be regarded as a British Standard. It will be withdrawn upon publication of its content in, or as, a British Standard.

The PAS process enables a specification to be developed rapidly in order to fulfil an immediate need in industry. A PAS may be considered for further development as a British Standard, or constitute part of the UK input into the development of a European or International Standard.

Use of this document

It has been assumed in the preparation of this PAS that the execution of its provisions will be entrusted to appropriately qualified and experienced people, for whose use it has been produced.

Presentational conventions

The provisions of this PAS are presented in roman (i.e. upright) type. Its methods are expressed as a set of instructions, a description, or in sentences in which the principal auxiliary verb is “shall”.

Commentary, explanation and general informative material is presented in italic type, and does not constitute a normative element.

Spelling conforms to The Shorter Oxford English Dictionary. If a word has more than one spelling, the first spelling is used.

Contractual and legal considerations

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with this PAS does not itself confer immunity from legal obligations.

This page deliberately left blank

Background

Increasingly, numerous biocidal products are displaying claims of residual antimicrobial activity (e.g. long-lasting protection, 24-h protection and residual action). With these emerging product claims and the impending Biocidal Products Regulation (BPR) which requires the submission of product dossiers to substantiate product claims, there is a need for a test method by which residual antimicrobial activity can be measured and assessed.

At present there is no European Standard test methodology for assessing the residual antimicrobial activity of a chemical disinfectant/antimicrobial product, therefore researchers and companies have designed test methods in an attempt to demonstrate residual antimicrobial efficacy and support these claims. However, these methods largely involve applying a product to a surface and leaving it for a defined period of time before challenging with micro-organisms. The limitation of such methods is that the surface remains undisturbed following application. In reality, consumer research (*Cleaning Behaviours in the Home* [1]) shows that when observing behavioural habits in the domestic environment or workplace, once a product has been applied to a surface, the surface is continually exposed to abrasion such as touching and wiping. This results in potential re-soiling and re-contamination of the surface before the next time a product is applied. Consumer research (*Cleaning Behaviours in the Home* [1]) also shows that the application of a product typically occurs every 24 h.

The test method in PAS 2424 has therefore been designed to reflect within a laboratory test method the actual conditions in which a product is designed to be used. It takes into consideration abrasion and re-contamination by including abrasion cycles and re-inoculations over a 24-h period and remains as close as possible to the practical conditions that are outlined in the current European Standards (e.g. test surface, contact times, micro-organisms, organic load, etc).

Overview of the test method in this PAS

a) An initial inoculum of bacteria or yeast is applied to a steel disc and allowed to dry. This initial step simulates a contaminated surface before the application of a disinfectant product.

b) A prepared sample of the test product (chemical disinfectant) is applied to the inoculated disc and allowed to dry. This gives a dried treatment which represents a surface that is likely to be exposed to abrasion and re-contamination before the next time it is treated with a disinfectant product.

c) Over a period of 24 h the disc undergoes a series of abrasion cycles and re-inoculations which are designed to simulate the abrasion and re-contamination (via touch and exposure) of a surface in between treatment with a disinfectant product.

d) The disc is exposed to a final inoculum challenge (24 h after product application) under defined conditions for a specified contact time. The final inoculum challenge simulates an event that will promote the application of a disinfectant product.

e) After the specified contact time the disc is transferred to a validated neutralizer solution so that the action of the residual disinfectant is quenched. The number of surviving organisms which can be recovered from the disc is determined quantitatively.

disinfectant is also determined. The reduction in viable counts as a result of the residual product is calculated by the difference. In order to demonstrate residual efficacy the product shall give a ≥3 log reduction when challenged 24 h after product application.

Relationship with other publications

PAS 2424 has been designed to take into consideration the experimental conditions set out in BS EN 1276:2009, *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2/step 1)* and BS EN 13697:2001, *Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2/step 2)*. These standards have been chosen because they are recognized test methods that contain experimental conditions representative of the conditions of use of chemical disinfectant products.

BS EN 13697:2001 involves applying an inoculum to a surface and allowing it to dry before applying a test product to the dried film for a specified contact time and transferring the surface to neutralization medium to quench the antimicrobial reaction. PAS 2424 is not designed to test a product at this initial stage but is intended to assess the residual efficacy of a product that has dried onto a surface which is subsequently exposed to abrasion.

Therefore, PAS 2424 has been designed as an extension of BS EN 13697:2001 and is intended to be used in conjunction with this recognized standard allowing BS EN 13697:2001 to assess the initial antimicrobial action of a product and PAS 2424 to assess the residual antimicrobial action.

It is for this reason that the results from PAS 2424 will only be considered if the product tested also achieves a pass according to BS EN 13697:2001 (≥4 log reduction) under conditions required for the product's application of use (e.g. specified contact times, micro-organisms, organic load, etc.).

Conclusion

PAS 2424 is regarded as a suitable solution for screening disinfectant products for their residual antimicrobial efficacy on a surface, and takes into consideration that surfaces are subjected to a degree of abrasion post-treatment. Ultimately, the test is designed to show how a disinfectant product will most likely perform in terms of its real usage, and will enable residual antimicrobial efficacy claims to be made about the product.

This document specifies a test method for determining whether a product does or does not have residual antimicrobial (bactericidal and/or yeasticidal) properties on hard, non-porous surfaces over a 24-h time period with abrasive action.

This laboratory test simulates practical conditions of application and therefore takes into account factors such as contact time, temperature, test organisms, test surface and interfering substance, i.e. conditions which may influence its action in practical situations.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. However, for some applications, the recommendations of use of a product may differ and therefore additional test conditions need to be used.

This PAS specifies a test method for residual bactericidal and/or yeasticidal activity of liquid, chemical disinfectant products that are applied to hard, non-porous surfaces which are likely to undergo abrasive action. The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used in accordance with BS EN 13697:2001.

It has been designed as an extension of BS EN 13697:2001 and is intended to be used in conjunction with this recognized standard allowing BS EN 13697:2001 to assess the initial antimicrobial action of a product and PAS 2424 to assess the residual antimicrobial action. Therefore the results from this PAS will only be considered if the product tested also achieves a pass according to BS EN 13697:2001 (≥4 log reduction) under conditions required for the test product's application of use (e.g. specified contact times, micro-organisms, organic load, etc.).

It is applicable to ready-to-use products or dilute-to-use products that form a homogeneous, physically stable preparation when diluted with hard water.

It is applicable to products that are used in areas that are covered in BS EN 13697:2001. This includes but is not limited to hard surfaces in the food industry, institutional areas such as schools, hospitals and nursing homes, in the workplace and in the home/domestic environment.

It is not applicable to thickened or viscous products such as toilet bleaches or gels, wash-off products and products that are permanently bound to a surface.

This PAS is designed for laboratories that perform antimicrobial testing in order to test the residual antimicrobial properties of liquid, chemical disinfectant products.

NOTE 1 The sticky nature of thickened products and gels make the abrasion regime difficult to perform and therefore could lead to unreliable results.

NOTE 2 The testing of wash-off products would require a method with the inclusion of a rinsing step.

NOTE 3 Intrinsically antimicrobial surfaces are designed to remain active for longer than the 24 h covered in this test method.

2 Normative references

The following referenced documents are necessary for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

BS EN 10088-1, *Stainless steels – Part 1: List of stainless steels*

BS EN 10088-2, *Stainless steels – Technical delivery conditions for sheet/plate and strip of corrosion resisting steels for general purposes*

BS EN 12353, *Chemical disinfectants and antiseptics – Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

BS EN 1276:2009, *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2/step 1)*

BS EN 13697:2001, *Chemical disinfectants and antiseptics – Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements without mechanical action (phase 2/step 2)*

BS EN 14885:2006, *Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics*

For the purpose of this PAS, where possible, the terms and definitions given in BS EN 14885:2006, *Chemical disinfectants and antiseptics – Application of European Standards for chemical disinfectants and antiseptics* have been selected.

3.1 Terms and definitions

For the purpose of this PAS, the following terms and definitions apply.

3.1.1 bactericide
product which kills vegetative bacteria under defined conditions

[SOURCE: BS EN 14885:2006, **3.2.1**]

NOTE The adjective derived from "bactericide" is "bactericidal".

3.1.2 chemical disinfectant
product that is capable of chemical disinfection

[SOURCE: BS EN 14885:2006, **3.1.3**]

NOTE "Chemical disinfection" is defined in BS EN 14885:2006 as a "reduction of the number of micro-organisms in or on an inanimate matrix, achieved by the irreversible action of a product on their structure or metabolism, to a level judged to be appropriate for a defined purpose".

3.1.3 neutralizer
chemical agent or formulation that suppresses the residual microbicidal activity of a product within a specific test but does not kill, inactivate or inhibit the test organisms

[SOURCE: BS EN 14885:2006, **3.3.5**]

3.1.4 product
chemical agent or formulation used as a chemical disinfectant or antiseptic

[SOURCE: BS EN 14885:2006, **3.3.6**]

3.1.5 residual bactericidal activity
capability of a product to continue to produce a reduction in the number of viable bacteria cells of relevant test organisms under conditions defined in this PAS

3.1.6 residual yeasticidal activity
capability of a product to continue to produce a reduction in the number of viable yeast cells of relevant test organisms under conditions defined in this PAS

3.1.7 test organism
strain of a micro-organism selected for testing products within a standardised test

[SOURCE: BS EN 14885:2006, **3.3.7**]

NOTE For the purpose of this PAS the term micro-organism includes vegetative bacteria and yeast.

3.1.8 yeasticide
product that kills yeasts under defined conditions

[SOURCE: BS EN 14885:2006, **3.2.16**]

NOTE The adjective derived from "yeasticide" is "yeasticidal".

3.2 Abbreviations

ATCC – American Type Culture Collection

NCPF – National Collection of Pathogenic Fungi

NCTC – National Collection of Type Cultures

A prepared sample of the test product (chemical disinfectant) is applied to a steel disc that has been inoculated with bacteria or yeast and allowed to dry under defined conditions. Over a period of 24 h, the disc undergoes a series of abrasion cycles and inoculations designed to simulate the abrasion and re-contamination of a surface in between product application. The disc is exposed to a final inoculum challenge (24 h after product application) under defined conditions for a specified contact time. After this contact time the disc is transferred to a validated neutralizer solution so that the action of the disinfectant is quenched. The number of surviving organisms which can be recovered from the disc is determined quantitatively.

The number of bacteria or yeast on a steel disc treated with hard water in place of disinfectant is also determined. The reduction in viable counts as a result of the residual product is calculated by the difference. In order to demonstrate residual efficacy the product shall give a ≥3 log reduction 24 h after product application.

NOTE A 24-h time period has been applied for the purpose of this test method as a result of consumer research: Cleaning Behaviours in the Home *[1], for the areas to which this PAS applies. Should other areas be explored, further research would need to be performed into the number of abrasions a specific surface is exposed to over the desired time period.*

5 Performance requirements

5.1 General

When tested according to the obligatory test conditions set out in this PAS, the product shall achieve a ≥3 log (99.9 %) reduction in viable counts in the presence of 3.0 g/l bovine albumin fraction V.

Products that are tested using this method shall also achieve a pass according to the criteria specified in BS EN 13697:2001. The results from PAS 2424 shall only be considered if the product tested has also achieved a pass according to BS EN 13697:2001 (≥4 log reduction) under conditions required for the product's application of use (e.g. specified contact times, micro-organisms, organic load, etc.).

5.2 Requirements for residual bactericidal activity

For a claim of residual bactericidal activity, activity shall be evaluated using the following strains: *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus hirae* and *Escherichia coli* at a temperature of 20 °C ± 2 °C and a final contact time of 5 min ± 10 s.

5.3 Requirements for residual yeasticidal activity

For a claim of residual yeasticidal activity, activity shall be evaluated using the following strain: *Candida albicans* at a temperature of 20 °C ± 2 °C and a final contact time of 15 min ± 10 s.

Obligatory test conditions shall be passed in order to claim residual performance using the test method in this PAS.

NOTE Where appropriate (for specific purposes) additional bactericidal/yeasticidal activity should be determined under other conditions (e.g. time, temperature, additional strains, interfering substances and number of abrasion cycles) in order to take into account intended specific use conditions.

6.1 Test organisms

The residual bactericidal activity shall be evaluated using the following strains as test organisms:

- *Pseudomonas aeruginosa* ATCC 15442/NCTC 13359;
- *Escherichia coli* ATCC 10536/NCTC 10418;
- *Staphylococcus aureus* ATCC 6538/NCTC 10788;
- *Enterococcus hirae* ATCC 10541/NCTC 13383.

The residual yeasticidal activity shall be evaluated using the following strain as a test organism:

- *Candida albicans* ATCC 10231/NCPF 3179.

For certain product applications, additional test organisms may be used. For example, this may include, but is not limited to:

- *Listeria monocytogenes* ATCC 35152/NCTC 7973;
- *Salmonella typhimurium* ATCC 13311/NCTC 74;
- (Methicillin resistant) *Staphylococcus aureus* MRSA/NCTC 13277.

NOTE If additional organisms are used, they should be incubated under optimal growth conditions (e.g. temperature, time, culture media and atmosphere) and noted in the test report.

6.2 Culture media and reagents

6.2.1 General

All reagents shall be of analytical grade and/or appropriate for microbiological purposes.

All media may be sourced from external suppliers (either dehydrated or pre-prepared) and shall be of analytical grade and suitable for microbiological use.

NOTE 1 To improve reproducibility, commercially available dehydrated material should be used for the preparation of culture media and the manufacturer's instructions should be rigorously followed.

NOTE 2 All weights of chemical substances given in the test method in this PAS refer to the anhydrous salts.

6.2.2 Water

The water shall be freshly distilled or de-ionized water, sterilized by autoclave (**7.24**) or membrane filtration at a maximum of 0.45 µm pore size (**7.18**). The water shall be free from substances that are toxic or inhibiting to micro-organisms.

NOTE If the water is sterilized during sterilization of the reagents, this is not necessary, e.g. if the water is used for preparation of culture media which is subsequently sterilized.