Australian Government
Department of Health
Therapeutic Goods Administration

TGA instructions for disinfectant testing

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TGA Health Safety Regulation

Contents

Introduction

The Australian Government is responsible for regulating the quality of therapeutic goods, including medicines. In respect of therapeutic goods other than medical devices, this is principally achieved by specifying ministerial standards for the goods which may relate to a range of matters (e.g. the quality of the goods and the procedures to be carried out in their manufacture), and otherwise by applying default standards specified in the international pharmacopoeias that are defined in the Act.

Therapeutic Goods (Standard for Disinfectants and Sanitary Products) (TGO 104) 2019 (the Order) is designed to support the quality, safety and efficacy of therapeutic goods that are disinfectants, sterilants and sanitary fluids and powders.

The Order does so in relation to disinfectants by, in part, specifying a number of important performance requirements. These performance requirements principally require that disinfectants comply with specified microbiological tests, such as the TGA Disinfectant Test, to support the claims for bactericidal activity. Additional testing is required where a claim is made for a disinfectant in relation to the product having a sporicidal, fungicidal, tuberculocidal, virucidal or other biocidal use.

This document sets out for the purpose of identifying and explaining these testing requirements, the TGA Disinfectant Test (Part 1) and specific testing requirements (Part 2), principally for the purposes of the Order.

A number of terms used in this document, for instance ‘sporicide’, ‘tuberculocide’ are defined in the *Therapeutic Goods Regulations 1990*. A link to these regulations on the Federal Register of Legislation can be found at https://www.legislation.gov.au/Details/F2019C00136.

Part 1—The TGA disinfectant test

1 Test

(1) A disinfectant must be tested at the dilution recommended by the manufacturer on the product label.

(2) The test consists of:

(a) the following steps, in the following order:

(i) challenging the diluted disinfectant with bacterial inoculum;

(ii) withdrawing a sample after a given time and culturing the sample in a suitable recovery medium;

(iii) (except for household/commercial grade disinfectants) after this sampling, challenging the mixture again by a second inoculum, and after a second interval again sampling for culturing.

(3) The sample referred to in (2) is to be considered to pass or fail the test according to the extent of growth shown in the two cultures sampled.

(4) The test may be performed:

(a) for a hospital grade disinfectant – either:

(i) without the addition of sterile yeast as an organic soil ("Option A", "clean conditions"), using the organisms identified in column 2 of Table 1 and with the number of challenges and inoculum density also identified in Table 1 for such disinfectants;

(ii) with the addition of sterile yeast as an organic soil ("Option B", "dirty conditions"), using the organisms identified in column 2 of Table 1 and with the number of challenged and inoculum density also identified in Table 1 for such disinfectants; or

(iii) both with and without the addition of sterile yeast as an organic soil, according to the use-situations advocated on the label of the product, ie when use in both clean (Option A) and dirty (Option B) conditions is claimed.

(b) for a household/commercial grade disinfectant, with the use of a nutrient broth as a simulated soil (Option C);

(5) The steps referred to in (2) must be undertaken in accordance with the instructions or requirements in sections 2 – 9 of this Schedule.

Table 1. Selection of test parameters for disinfectants using the TGA Disinfectant Test.

Class of product	Organisms used in the test	Test option for resuspension of centrifuged organisms	Number of challenges	Inoculum density
Hospital grade disinfectant	*Ps. aeruginosa* *Pr. Vulgaris* *E. coli* *S. aureus*	Option A ("clean" conditions) Option B ("dirty" conditions")	2	$2x10^8$ - $2x10^9$
Household/commercial grade disinfectant	*E. coli* *S. aureus*	Option C	1	$2x10^8$ - $2x10^9$

2 Media

All media must be contained in capped glass containers and, when stored, the containers must be sealed tightly or refrigerated.

2.1 Sterile hard water

2.1.1 Dissolve 0.304g anhydrous calcium chloride and 0.065g anhydrous magnesium chloride in glass-distilled water, and make up to one litre.

2.1.2 Dispense into glass containers and sterilize by autoclaving at 121° ±1° C for 15 minutes.

Note: The AOAC Hard Water formulation[1] may be used.

2.2 Yeast suspension

2.2.1 Weigh 200g of moist compressed baker's yeast. Cream by the gradual addition of sterile hard water using a heavy glass rod for stirring. Decant the creamed portion into a flask, add more water to any lumpy residue remaining and repeat the creaming and decantation until no residue remains and 500ml of water has been used.

2.2.2 Shake the contents of the flask vigorously and strain through a 100-mesh sieve, breaking down any remaining lumps.

2.2.3 Add 500ml sterile hard water, shake vigorously and adjust the pH to 6.9-7.1 with 1N Sodium hydroxide.

2.2.4 Transfer 50ml, 100ml or 200ml of the yeast solution into screw-capped bottles.

[1] US EPA Standard Operating Procedure for Preparation of AOAC and OECD hard water and other diluents for preparation of antimicrobial products SOP Number: MB-30-01 Date Revised: 02-03-17 https://www.epa.gov/sites/production/files/2018-01/documents/mb-30-01.pdf

2.2.5 Autoclave at 121° ±1° C for 15 minutes and allow the autoclave to cool without releasing pressure. Store cold but not freezing.

2.2.6 Dry two Petri dishes to constant weight. Into each, pipette 25ml of sterilised yeast suspension, and dry to constant weight at 100°C. Calculate the average solids content of the suspension.

2.2.7 Before use, pipette 25ml of the sterilised yeast suspension into a beaker. Determine the pH using the glass electrode, and determine the volume of 1N sodium hydroxide solution needed to adjust the pH to within the range 6.9 to 7.1.

2.2.8 Immediately before use, add to each bottle of sterilised yeast, a volume of sterile hard water and a volume of 1N sodium hydroxide calculated to adjust the concentration of dry yeast to 5.0% and the pH to within the range 6.9-7.1. Discard prepared yeast 3 months after preparation.

2.3 Medium for growth of test organisms

2.3.1 Prepare a 10% w/v dextrose solution in distilled water, and sterilise by autoclaving at 121° ±1° C for 15 minutes. Cool to room temperature.

2.3.2 Prepare Wright and Mundy medium following the author's procedure (referred to in item 10(2)) or from a commercial product of the same composition (as referred to in item 11(b)) and sterilise by autoclaving at 121° ±1° C for 15 minutes. Cool to room temperature.

2.3.3 To each litre of Wright and Mundy medium prepared in item 2.3.2 add 10ml sterile dextrose solution prepared in item 2.3.1.

2.3.4 Aseptically dispense in either 10ml or 15ml amounts, as preferred.

2.3.5 This medium is referred to as ***Wright and Mundy dextrose medium***. AOAC Synthetic Broth[2] may also be used.

2.4 Recovery medium

2.4.1 Prepare nutrient broth as follows or from a commercial product of the same composition (as referred to in item 11(b)):

Add the following to 970ml of water and dissolve by heating.

Beef Extract Powder	10g
Peptone	10g
Sodium Chloride	5g

Adjust the pH to 8.0-8.4 using 1N Sodium Hydroxide.

Boil for 10 minutes and filter. Cool.

2.4.2 To each litre of nutrient broth solution prepared in item 2.4.1 add 30g polysorbate 80 (as referred to in item 11(b)).

2.4.3 Adjust pH to 7.2-7.4 using 1N Sodium hydroxide.

[2] Synthetic Broth, AOAC is a chemically defined medium recommended by AOAC for growing inoculum, making subcultures and preparing various dilutions while testing disinfectants.

2.4.4 Autoclave at 121° ±1° C for 15 minutes, and immediately shake well to disperse the polysorbate 80.

2.4.5 Dispense aseptically in 10ml amounts into sterile capped glass tubes.

3 Test inoculum

3.1 Test organisms

The following 4 organisms are to be used for hospital grade disinfectants for Option A or Option B.

Pseudomonas aeruginosa	NCTC 6749;
Proteus vulgaris	NCTC 4635;
Escherichia coli	NCTC 8196; and
Staphylococcus aureus	NCTC 4163.

The following 2 organisms are to be used for household/commercial grade disinfectants for Option C:

Escherichia coli	NCTC 8196; and
Staphylococcus aureus	NCTC 4163.

3.2 Preparation of inoculum

3.2.1 Incubate the contents of an ampoule of freeze-dried culture overnight at 37° ± 1°C in Wright and Mundy dextrose medium.

3.2.2 Inoculate the incubated culture onto nutrient agar slopes in McCartney bottles. Store for up to 3 months at 4° ± 1°C.

3.2.3 At a suitable period before the test is to be conducted, sub-culture from an agar slope into 10ml or 15ml quantities of Wright and Mundy dextrose medium. Incubate at 37° ± 1°C for 24 ± 2 hours.

3.2.4 Sub-culture from the medium in item 3.2.3 into fresh medium, using an inoculating loop of 4mm in diameter. Incubate at 37° ± 1°C for 24 ± 2 hours.

3.2.5 Repeat item 3.2.4 daily. For the test procedure use only those cultures which have been sub-cultured at least 5 times, and not more than 14 times.

3.2.6 Filter test cultures of *P. aeruginosa* and *S. aureus* through sterile Whatmans No. 4 filter paper.

3.2.7 Centrifuge all test cultures until cells are compact, and remove supernatant with a Pasteur pipette.

3.2.8 Resuspend test organisms in the original volume of liquid (i.e. 10ml or 15ml), and shake for 1 minute with a few sterile glass beads.

3.2.8.1 For **Option A**, resuspend in sterile hard water.

3.2.8.2 For **Option B**, resuspend in a mixture of 4 parts yeast suspension (prepared as in item 2.2) to 6 parts sterile hard water.

3.2.8.3 For **Option C**, resuspend in nutrient broth (prepared as in items 2.4.1 and 2.4.3 and sterilised by autoclaving).

3.3 Enumeration of inoculum

Immediately before testing, sample the resuspended inoculum and enumerate using 10-fold dilutions in quarter-strength Ringer's solution and the pour-plate technique. The number subsequently counted must represent not less than 2×10^8 or more than 2×10^9 organisms per millilitre or the test is considered invalid. Retain tube containing 10^{-7} dilution for use in controls (items 7.3 and 7.4).

4 Disinfectant dilutions

Quantitatively dilute a sample of the disinfectant to the specified extent, using sterile hard water as diluent. Use not less than 10ml or 10g of sample for the first dilution, and not less than 1ml of any dilution to prepare subsequent dilutions. Make all dilutions in glass containers on the day of testing. The glass containers must be twice rinsed in glass-distilled water, and sterilised.

5 Temperature

Where air-conditioning does not maintain test solutions at 21° ± 1°C, hold the containers in which the test is to be carried out in a waterbath at this temperature.

6 Test procedure

Perform the following test, using each of the four test organisms (item 3.1), except where the Standard directs otherwise. It is not necessary to test with all organisms simultaneously.

6.1 Add 3ml of diluted disinfectant to a capped glass container.

6.2 Start a timing device. Immediately inoculate disinfectant with 1ml of culture (prepared in item 3.2) and mix by swirling.

6.3 At 8 minutes, subculture one drop (0.02ml ± 0.002ml) into each of 5 tubes containing recovery broth. To ensure delivery of 0.02ml into the first tube of recovery broth at exactly 8 minutes, it will be necessary to withdraw a suitable amount from the disinfectant test mix shortly beforehand. This must be immediately preceded by vortexing. Surplus sample must be returned to the test mix (refer to item 11(d)).

6.4 At 10 minutes, inoculate disinfectant with a further 1ml of culture, and mix by vortexing.

6.5 At 18 minutes, proceed as in item 6.3.

6.6 Mix the contents of all tubes of recovery broth by vortexing. Incubate at 37° ± 1°C for 48 ± 2 hours.

6.7 Examine for growth and record results.

6.8 For each test organism repeat steps 6.1-6.7 on each of 2 subsequent days, using a fresh disinfectant dilution and a freshly prepared bacterial suspension.

7 Controls

7.1 Recovery broth contamination

Incubate one uninoculated tube of recovery broth at 37° ± 1°C for 48 ± 2 hours and examine for growth. If growth occurs, the test is considered invalid due to contamination of the recovery broth.

7.2 Disinfectant contamination

To 1 tube of recovery broth, add 0.02ml of diluted disinfectant. Incubate at 37° ± 1°C for 48 ± 2 hours. If growth occurs, the test is considered invalid. Growth in item 7.2 but not item 7.1 indicates contamination of the disinfectant test solution.

7.3 Fertility test

To 1 tube of recovery broth, add 1.0ml of the 10^{-7} dilution retained in item 3.3. Incubate at 37° ± 1°C for 48 ± 2 hours and examine for growth. If no growth occurs, the test is considered invalid.

7.4 Inactivator efficacy

To 1 tube of recovery broth, add 0.02ml of diluted disinfectant and 1.0ml of the 10^{-7} dilution retained in item 3.3. Incubate at 37° ± 1°C for 48 ± 2 hours, and examine for growth. If no growth occurs, the test is considered invalid. Growth in item 7.3 but not in item 7.4 indicates inadequate inactivation of the disinfectant.

8 Procedure in case of invalid controls

When any control renders the test invalid, the test is to be repeated. Fresh recovery broth is to be used if growth occurred in control item 7.1, or if no growth occurred in controls items 7.3 or 7.4.

Should disinfectant contamination be indicated by control item 7.2 on both occasions, the disinfectant is considered to fail the test. Should inadequate inactivation of the disinfectant be indicated by control item 7.4 on both occasions, the test is considered invalid (refer to item 11(c)).

9 Results

The dilution test is considered to pass the test if there is no apparent growth in at least two out of the five recovery broths specified in item 6.3 and no apparent growth in at least two of the five recovery broths specified in item 6.5 on all three occasions, using all four organisms.

Part 2 - Disinfectant performance tests

Division 1 – Tests for the physical stability, chemical stability, and shelf life of disinfectants

1 Physical stability

Physical stability testing may include:

(a) appearance (e.g. emulsion stability, clarity etc.)

(b) odour

(c) pH

(d) immediate container and product compatibility

2 Chemical stability

Chemical stability testing must comply with the following:

(a) active(s), to be determined by an assay that is valid for the active (eg. HPLC, GC, titration),

(b) active(s) to be above the minimum level required to pass the appropriate TGA test(s) over the shelf life, and

(c) all chemical testing to be carried out on duplicate samples.

3 Antimicrobial stability

A disinfectant must pass at least one suitably sensitive test according to the appropriate evaluation guidelines for the level/grade of disinfectant (see table below) at the final end point with the levels of active(s) at the final predicted final level.

Disinfectant	Appropriate test
Hospital Grade	Carrier test[3] (with or without soil as appropriate)
Household/Commercial Grade	The TGA disinfectant Test in Part 1, under the conditions in Option C, or carrier test (with organic soil)

[3] Disinfectants are used on contaminated surfaces, thus making it necessary to evaluate their microbicidal action on representative carrier materials contaminated with a dried challenge microorganism(s). These tests are more stringent than suspension tests, such as the TGA Disinfectant Test. Information on the appropriate carrier test is provided in Division 2, section 2.

4 Stability testing frequency

Accelerated studies

Suggested testing intervals are:

0, 3, 6 and 12 months, but other time points e.g. 1, 2, or 5 months may also be used and may be necessary for adequate accelerated studies. The minimum active concentration level from which the shelf life is predicted from accelerated data must be no lower than the minimum active concentration required to pass the appropriate microbial test.

Real time studies

Testing at initial and annual intervals should be sufficient. However, it is not necessary to have completed these testing programs before launching the product. Samples from production should undergo real time testing. These samples should be monitored over the shelf life of the product as proposed by the sponsor.

5 Packs for stability testing

Stability testing should be carried out on product stored in the proposed packaging material, and in the case of real time testing, this requirement is mandatory.

6 Prediction of shelf life

Shelf life may be predicted from accelerated data only if the accuracy and reproducibility of the results is adequate to support the extrapolation. All data must be fully defensible.

Extrapolation at various times and temperatures may be determined according to the following general rules:

Elevated Temp[a] above storage conditions[b]	Time Period	Possible Shelf Life Prediction
+10°C	3 months	1 year
+15°C	3 months	18 months
+10°C	6 months	2 years
+15°C	6 months	3 years
+10°C	9 months	3 years

(a) incubator temperatures must be monitored and logged

(b) storage conditions for Australia are considered to be the temperatures of 25 - 30°C. The reference temperature used for stability testing will be that on the label.

Alternative predictive models/rules for extrapolation can be used, however, after proper scientific validation.

At least four real time data points, including the initial and three months, should be evaluated using acceptable statistical methods to justify the extrapolation.

The predicted value of the final concentration of the disinfectant active should not fall below the 90% limit of the label claim at the end of the extrapolated shelf life.

The stability programme should collect real time data from a production batch stored at the recommended storage condition. This should be used to confirm the extrapolated shelf life.

The TGA should be advised if the product falls outside specification with appropriate action, commensurate with public safety, negotiated and agreed.

If there are minor changes to excipients, product stability should be confirmed, together with a single microbial efficacy test as for the final determination of shelf life, unless justification is provided using a risk-based approach.

Division 2 – Microbial efficacy general requirements

1. General requirements

All tests should be carried out by a GMP licensed laboratory or laboratory accredited to ISO/IEC 17025 or equivalent eg, NATA, TGA, US FDA, PIC/S, US EPA, NAMAS UK etc. Data sourced from non-accredited laboratories will only be accepted in relation to testing conducted using blood borne viruses, including HIV, Hepatitis B and C, Ebola and other haemorrhagic viruses.

Where international standards and test methods (CEN, AOAC, US EPA, ASTM) are referenced in testing, testing should be conducted against the current and relevant standard at the time the testing was conducted, and the edition number of the standard clearly stated within the testing results. Where testing has been conducted to a more recent standard than those set out below, the more recent standard will be acceptable.

Regardless of whether the product is used undiluted or diluted by the user to the Minimum Recommended Concentration, the product should be formulated at the lower extreme of the active ingredient in the product specification, stored to expiration and then subjected to the microbial efficacy tests.

The product may be tested before the end of the shelf life to initially provide a full set of data for evaluation purposes if product stored to the end of the expiration period is not available. The data should be generated from more than one batch of product (preferably three batches). Testing at the end of the shelf life will then need to be performed according to the microbiological stability requirements specified above, when product stored to the end of the expiration period becomes available. End of shelf life data should be generated from a minimum of two batches.

A common approach to developing a worst case product for testing is to use accelerated life testing to predict the final level of actives. These levels (with a safety factor) are then used in the formulation of a sample for efficacy testing. However, some products are unstable at elevated temperatures and may not be suitable for accelerated stability testing. In general, real time stability studies are preferred.

Data from all tests specified for each grade and level of disinfectant is not required for monitoring of stability (see table above for the appropriate tests to be used to generate stability data). Tests must be carried out at the pH, temperature and time (unless the stability studies are conducted under the accelerated conditions) recommended on the label for the use, or each level of use where there is more than one level.

Test methods must be validated by individual laboratories for each test method used in accordance with tests which have been validated or refereed at national or international level. This should involve validation of individual operators by performance of the specified test using a control or product that is known to pass the specified test at a given concentration as well as testing to determine the most appropriate neutraliser/inactivator, if necessary.

2. Specific tests for hospital grade disinfectants and household / commercial grade disinfectants

Hospital grade disinfectants

Bactericidal efficacy (excluding tuberculocidal) is the only mandatory requirement for a hospital grade disinfectant without specific claims.

Bactericidal testing

These disinfectants MUST pass Option B of the TGA Disinfectant Test under dirty conditions. This is a semi-quantitative suspension test. Testing in accordance with the dirty conditions option of EN 13727[4] will also be accepted. Other suspension tests may be acceptable if modified to include 5% organic soil and water of minimum hardness >340ppm.

If the disinfectant is clearly labelled for use on a pre-cleaned surface, Option A of the TGA Disinfectant Test may be used. Under these circumstances, the clean conditions option of EN 13727 will also be accepted as an evaluation test. Other suspension tests may be acceptable under clean conditions provided hard water (>340 ppm) is used.

If specific label claims are made for vegetative bacterial species other than those covered by the required test organisms, the testing above must be conducted using these additional organisms. Specific testing as described below must be conducted when label claims are made for activity against mycobacteria and spore-forming organisms.

These disinfectants MUST also pass a bactericidal carrier test. AOAC[5,6] methodology (60 carriers per organism) or equivalent method, such as ASTM E2197[7] or ASTM E2111[8] may be used. Testing conducted in accordance with EN 14561[9] or EN 13697[10] will be accepted if modified to use 60 carriers per test organism. If the AOAC Use Dilution Test is chosen, the disinfectant passes when there is no growth in 59 out of 60 carriers per organism. If the Hard Surface Carrier Test is used, the criteria for pass or fail are described in the test method. If 10 carriers are used for additional claims, no carriers may show growth. If the ASTM or other methods are used, the test organisms and test criteria as described in the AOAC Hard Surface Carrier Test apply.

If additional claims regarding organisms not included in the original test are to be made (e.g. "Kills E coli O157), the extra organism(s) can be tested using 10 carriers rather than 60. Soil should be included at a minimum of 5% blood serum and inorganic soil such as hard water at a minimum of 340ppm.

If any specific biocidal claims are made (i.e., virucidal, fungicidal, tuberculocidal, sporicidal, or other biocidal activity), the disinfectant must pass appropriate tests as specified below. All tests should be carried out using the exposure time, temperature and pH specified on the label. For products intended for use on surfaces that have not been pre-cleaned, 5% organic soil must be included. For products that are intended for use on surfaces that have been pre-cleaned, organic soil need not be added to the test, with the exception of testing against blood borne viruses, as discussed in the virucidal claim section below.

[4] European Standard EN 13727 *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity in the medical area – Test method and requirements (phase 2, step 1)*
[5] *Official Methods of Analysis of the AOAC International*, current edition, Use-Dilution Method 955.15 and 964.02
[6] *Official Methods of Analysis of the AOAC International*, current edition, Hard Surface Carrier Test Methods
[7] ASTM E2197 *Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides*
[8] ASTM E2111 *Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal and Sporicidal Potencies of Liquid Chemicals*
[9] European Standard EN 14561 *Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area - Test method and requirements (phase 2, step 2)*
[10] European Standard EN 13697 *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)*

Fungicidal testing

For a fungicidal claim, any test of reasonable scientifically-based, peer reviewed methodology will be considered, although carrier test methodology is preferred. Acceptable tests include the AOAC Use Dilution Test, which can be modified for fungi, with 10 carriers for each of two batches of product, and acceptance criteria of all fungal spores on all carriers killed, the AOAC Fungicidal Test[11], which can be modified for a carrier test, and the ASTM E2197 test methodology, with acceptance criteria as for the modified AOAC Use Dilution Test. Tests conducted in accordance with EN 13624[12] or EN 13697[10]1010 will also be accepted.

Virucidal testing

For a general virucidal claim, not including blood borne viruses such as HIV, HBV, HCV, Ebola etc., the disinfectant MUST pass tests, using Poliovirus/Parvovirus and Herpes simplex as the test viruses. Testing to support label claims against HIV, Hepatitis B (HBV), Hepatitis C (HCV), must use 50% whole blood as added soil.

The tests may be suspension tests but carrier tests are preferred. Methods that may be used as a basis are the AOAC Use Dilution Test modified for viruses and ASTM E2197[11]. One surface is required to be tested for each of two batches of product. Guidance on carrier test methodology is provided in ASTM E 1053[13]. Guidance on suspension test methodology is provided in ASTM E 1052[14]: *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension*. If a suspension test is used, the methodology of EN 14476[15] is acceptable, if the test is conducted under dirty conditions and if Herpes simplex is used in addition to the organisms required by the standard.

NOTE: The World Health Organisation (WHO) is conducting a global campaign aimed at the eradication of Polio. As a result, there will be restrictions on the use of Poliovirus (both wild and vaccine strains), in the laboratory. For this reason, other viruses may be accepted for demonstration of virucidal efficacy. Possible alternatives include Hepatitis A, Feline Calicivirus or Murine Norovirus, however, any company choosing a virus other than Poliovirus/Parvovirus for virucidal testing should justify the use of an alternative virus - the justification should include evidence that the virus chosen is of equivalent resistance in in-vitro testing.

Viral recovery systems that may be used include tissue culture, embryonated egg and animal inoculation.

Tests on the designated prototype viruses should be performed in quadruplicate against a recoverable viral titre of at least 4-$\log_{10}$, which must be recoverable from the test surface or suspension, and should show complete viral inactivation. If cytotoxicity is apparent, a 3-$\log_{10}$ reduction must be demonstrated beyond the cytotoxic level and there should be complete viral inactivation. Cytotoxicity is more easily overcome in suspension tests, for which there should be

[11] *Official Methods of Analysis of the AOAC International*, current edition, Fungicidal Activity of Disinfectants Method 955.17
[12] European Standard EN 13624 *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area – Test method and requirements (phase 2, step 1)*
[13] ASTM E 1053 *Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces*
[14] ASTM E 1052 *Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension*
[15] European Standard EN 14476 *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area – Test method and requirements (phase 2, step 1)*

a 4-$\log_{10}$ reduction with complete viral inactivation. Suitable controls should be employed, which include:

- cytotoxicity controls,
- disinfectant neutralization controls,
- quantitative viability control,
- cell control, and
- carrier wash-off control.

If a label claim against HIV, Hepatitis B (HBV), Hepatitis C (HCV) or other specific virus is made, separate data must be provided, in addition to the above. Suspension tests may be used for additional specific viral claims, but the recoverable viral challenge should reflect the titre found in the clinical situation. Complete viral inactivation is preferred, with a minimum 4-log reduction of specific viruses. For HIV, the method should be based on the principles described above. The use of a surrogate virus for HIV claims is not permitted.

For viruses that cannot be cultured, surrogate viruses may be used. For HBV, tests with a surrogate organism such as Duck Hepatitis B Virus (DHBV) are acceptable. The electron microscope viral disruption and antigenicity tests are NOT acceptable, as they are not reliable indicators of HBV infectivity. A suitable surrogate for HCV may be Bovine Viral Diarrhoeal Virus (BVDV). Tests for HIV and HCV MUST be conducted against cell-associated virus – a suspension test may be used if necessary. Organic soil for tests against HIV and surrogates for HBV and HCV and Ebola MUST be a minimum of 50% whole blood. Suitable references for test methods are Murray (1991)[16], Druce (1995)[17], and Lavelle (1987)[18].

With regard to testing against HIV, there can be difficulties associated with testing using cell-associated virus. Non-cell associated virus can be used for HIV testing of hospital or household grade disinfectants not intended for use on medical devices if:

- a carrier test is used instead of a suspension test;
- the minimum level of organic soil used in the test is 50% whole blood; and
- the product is clearly labelled for use on a pre-cleaned surface.

There are a number of other viruses which cannot be cultured which may be the subject of label claims. These include Norwalk virus and Norovirus. There are also viruses such as SARS associated coronavirus, which can be cultured but may not be suitable for use in test laboratories due to biosecurity issues. Feline calicivirus has been accepted as a surrogate to justify claims against Norwalk and Noroviruses. For claims against SARS or COVID-19, Human coronavirus 229E or Murine hepatitis virus can be used as a surrogate if either the SARS virus or the COVID-19 virus cannot be used.

Other strains of Human coronavirus will also be considered if Human coronavirus 229E cannot be used.

[16] Murray SM, JS Freiman, K. Vickery, D.Lim, YE Cossart, RK Whiteley "Duck Hepatitis B Virus: a model to assess efficacy of disinfectants against hepadnavirus activity". *Epidemiol Infect* (1991) 106, 435 – 443
[17] Druce JD, D Jardine, SA Locarnini, CJ Birch "Susceptibility of HIV to inactivation by disinfectants and ultraviolet light" *Journal of Hospital Infection* (1995) 30 pp
[18] Lavelle George C, "Virucidal activity of Disinfectants: Predicting and Assessing Product Efficacy" *Chemical Times and Trends*, January 1987 45-50

In the event that Human coronavirus or Murine hepatitis virus cannot be used, consideration will be given to use of other animal coronaviruses. Viruses that have been suggested include Bovine coronavirus and Feline coronavirus. It is recommended that TGA be contacted if these viruses are to be used (at devices@tga.gov.au). If virucidal testing is limited to lipid/enveloped viruses, such as Herpes simplex virus, a label claim for general virucidal activity will not be permitted. The label must reflect the specific viruses used for the limited testing.

Tuberculocidal/mycobactericidal testing

For a tuberculocidal claim, results from a quantitative carrier test should be provided. A variety of organisms may be used, such as:

M bovis	*(BCG)*
M tuberculosis	*H37RV*
M terrae	*ATCC 15755*

NOTE: *M smegmatis* is NOT acceptable as this organism is comparatively easy to kill and its resistance patterns are substantially different from *M bovis* etc.

The acceptance criterion is a 6-$\log_{10}$ reduction in test organisms. If the carrier test used is not quantitative (e.g. the AOAC *Test for Tuberculocidal Activity of Disinfectants*[19]), a suspension test may be used to demonstrate a 6-log reduction.

Appropriate tuberculocidal tests that may be modified in line with points raised above include:

- the AOAC *Test for Tuberculocidal Activity of Disinfectants* (with the exception of the in-vitro screening test using *M smegmatis*);
- the EPA Quantitative Tuberculocidal Activity Test[20];
- ASTM E2111[8];
- ASTM E2197[7];
- the Ascenzi test[21]; and
- EN 14348[22]

Sporicidal testing

For a sporicidal claim, a carrier test or a suspension test may be used. The results should show a 6-$\log_{10}$ reduction in spores. However, if the AOAC Sporicidal Test[23] is used, growth is allowed from two carriers or less. Other carrier tests that may be suitable are those performed by the

[19] *Official Methods of Analysis of the AOAC International*, current edition, Tuberculocidal Activity of Disinfectants Method 965.12
[20] US EPA *Standard Operating Procedure for Quantitative Suspension Test Method for Determining Tuberculocidal Efficacy of Disinfectants Against Mycobacterium bovis (BCG)*, SOP Number: MB-16-01, 2009
[21] Ascenzi JM, RJ Ezzell, TM Wendt, "A More Accurate Method for Measurement of Tuberculocidal Activity of Disinfectants" *Applied and Environmental Microbiology*, Sept 1987 p2189-2192
[22] European Standard EN 14348 *Chemical disinfectants and Antiseptics – Quantitative Suspension Test for the Evaluation of Mycobactericidal Activity of Chemical Disinfectants in the Medical Area including Instrument Disinfectants – test methods and requirements (phase 2, step 1)*
[23] *Official Methods of Analysis of the AOAC International*, current edition, Sporicidal Activity of Disinfectants Test – Method II, Method 966.04

Hospital Infection Research Laboratory at Dudley Road Hospital, Birmingham, UK[24], and tests conducted in accordance with ASTM E2197[7], with acceptance criteria as for the AOAC Sporicidal Test. The methodology of EN 17126[25] will be accepted, if the test is modified to show a 6-$\log_{10}$ reduction in spores over the labelled exposure time.

Suitable test organisms include:

Clostridium sporogenes	ATCC 3584
Bacillus subtilis	ATCC 19659 or NCTC 10073

Label claims against *Clostridium difficile* can only be made if the methodology uses spores. The claim cannot be made if a vegetative form of *C. difficile* is used.

Hospital grade disinfectant wipes

The following tests are intended to apply to products making claims of surface disinfection. They are not intended to apply to products claiming activity solely within the wipe.

A hospital grade disinfectant wipe is required to:

(1) Pass a bactericidal suspension tests, such as the TGA Test (Option B for dirty conditions, Option A for clean conditions), or EN 13727. The suspension test chosen should be conducted on the product after extraction from the wipe. Testing in accordance with the European Standard EN 16615[25] will also be accepted.

(2) Alternatively, a modified AOAC Germicidal Spray Test[26] may be used, with 60 carriers, tested by wiping the surface of the carriers with the saturated wipe and subculturing the carriers after the specified contact time. One wipe should be used for a minimum of 10 carriers. The performance criteria should be no growth from 59/60 carriers.

(3) If a suspension test is used, the product must pass a simulated in-use test showing that the efficacy of the disinfectant is not reduced when combined with a wipe. Organic soil at a minimum of 5% blood serum must be included for products used under dirty conditions. The design of the test should be based on a carrier/surface test and could involve wiping the wipe over a carrier/surface and culturing both the carrier and the liquid that has been expressed from the used wipe. Alternatively, the entire wipe could be cultured.

(4) Any test of reasonable design employing these principles may be acceptable. At least 60 carriers/surfaces should be used per organism tested with 59/60 carriers showing no growth. The US EPA document "Product Performance Test Guidelines[27]" provides guidance on acceptable test methodology.

(5) If specific biocidal claims are made, all claims should be supported with data from a suspension or carrier test, with added soil as appropriate. These tests should be carried out according to the requirements of hospital grade disinfectants as detailed above. The simulated in-use test can be carried out using the organism from the most stringent claim. This is in addition to verifying the most stringent claim with a carrier or suspension test.

[24] "Sporicidal Activity of Glutaraldehyde and Hypochlorites" *Journal of Hospital Infection*, (1980) 1 63-75
[25] European Standard 17126 Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area. Test method and requirements (phase 2, step 1)
[26] European Standard EN 1276 *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*
[27] United States Environmental Protection Agency "Product Performance Test Guidelines" OCSPP 810.2200: *Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations,* 2012

(6) Where a wipe is reusable, the wipe must pass a simulated in-use test after the product has been subjected to a re-use protocol. Any reasonably designed test will be considered – a suggested method would include challenging the wipe by wiping it on a variety of surfaces. These surfaces should have been subject to periodic contamination with a microbiological bioburden at a concentration of 10^6 CFU/5mL use solution. The wipe should be allowed to dry between uses. At the end of the use period, the product should be subjected to a simulated in-use test as described in clause 3 above.

Hospital grade disinfectant wipes and sponges where the ingredient that is active cannot be expressed

Any reasonably designed test will be considered – a suggested method would include challenging the wipe or sponge by wiping it on a variety of surfaces. These surfaces should have been subject to periodic contamination with a microbiological bioburden. The wipe or sponge should be allowed to dry between uses. At the end of the use period, the product should be subjected to a simulated in-use test as described in clause 3 of "Hospital grade wipes." If claims against specific bacteria are made, then these organisms should be included in the test. Testing in accordance with EN 16615[28] will also be accepted.

Hospital grade disinfectant surface sprays

A hospital grade disinfectant that is a surface spray disinfectant is required to:

(1) Pass a bactericidal carrier test such as the AOAC Germicidal Spray Test. Testing in accordance with EN 14561 or EN 13697 will be accepted (test to be chosen depends on intended use – for hospital grade disinfectants, EN 14561 is the most appropriate). Sixty carriers per organism should be used, plus 10 carriers for each additional bactericidal claim. The performance criteria should be no growth from 59/60 carriers.

(2) Pass the tests specified in clause 1 with added organic and inorganic soil (if the product is for dilution). Organic soil should be a minimum of 5% blood serum and inorganic soil should be hard water (minimum hardness 340ppm). For products intended for use on pre-cleaned surfaces, organic soil need not be included. A surface spray disinfectant should be tested against *Salmonella enterica* serotype *choleraesuis* (*Salmonella choleraesuis*), *Staphylococcus aureus* and *Pseudomonas aeruginosa* if the AOAC Germicidal Spray Test is used. If other tests are chosen, it is expected that organisms similar to these would be used.

(3) Pass carrier tests as described in previous clauses, if specific biocidal activities are claimed. Inorganic and organic soil should be included, unless the product is clearly labelled for use on pre-cleaned surfaces.

Household/commercial grade disinfectants

Household/commercial grade disinfectants must pass Option C of the TGA Test, or a test conducted in accordance with EN 1276[29], OR

[28] European Standard EN 16615 Chemical disinfectants and antiseptics. Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test). Test method and requirements (phase 2, step 2)
[29] European Standard EN 13697 *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)*

(1) Pass an appropriate bactericidal carrier test, using AOAC methodology (60 carriers per organism), EN 13727 or EN 13697[30] (using 60 carries per test organism). Organic soil is not necessary but inorganic soil such as hard water of minimum hardness 340ppm should be included. 10 carriers should be used for each additional organism.

(2) Pass tests as for hospital grade disinfectants where specific biocidal activities are claimed. Inorganic soil should be included. Note: If sporicidal claims are made, testing in accordance with EN 13704 Chemical disinfectants - Quantitative Suspension Test for the Evaluation of Sporicidal Activity of Chemical Disinfectants used in Food, Industrial, Domestic and Institutional areas - test method and requirements (phase 2, step 1)[31] will be accepted.

Household/commercial grade disinfectants wipes

The following tests are intended to apply to products making claims of surface disinfection. They are not intended to apply to products claiming activity solely within the wipe.

A household/commercial grade disinfectant wipe is required to:

(1) Pass the TGA Test Option C or testing in accordance with EN 13727 or EN 1276[32]. The test should be conducted on the product after extraction from the wipe. Organic soil is not necessary but inorganic soil such as hard water of minimum hardness 340ppm should be included. Alternatively, a modified AOAC Germicidal Spray Test as described for hospital grade disinfectants may be used. Testing in accordance with EN 16615 will also be accepted.

(2) If a suspension test is used, the product must also pass a simulated in-use test showing that the efficacy of the disinfectant is not reduced when combined with a wipe. Organic soil need not be included unless specific instructions for use on soiled surfaces are included on the label. The test method should be based on that described in clause 3 of "Hospital grade wipes". At least 60 carriers/surfaces should be used per organism tested, with 59/60 carriers showing no growth.

(3) If specific biocidal claims are made, all claims should be supported with data from an appropriate suspension or carrier test. These tests should be carried out as described for other household/commercial grade disinfectants. The simulated in-use test can be carried out using the organism from the most stringent claim. This is in addition to verifying the most stringent claim with a carrier or suspension test.

(4) Where a wipe is reusable, the wipe must pass a simulated in-use test after the product has been subjected to a re-use protocol. Any reasonably designed test will be considered – a suggested method would include challenging the wipe by wiping it on a variety of surfaces. These surfaces should have been subject to periodic contamination with a microbiological bioburden at a concentration of 10^6 CFU/5mL use solution. The wipe should be allowed to dry between uses. At the end of the use period, the product should be subjected to a simulated in-use test as described in clause 3 of "Hospital grade wipes".

[30] *Official Methods of Analysis of the AOAC International*, current edition, Germicidal Spray Products as Disinfectants, Method 961.02

[31] European Standard EN 13704 *Chemical disinfectants - Quantitative Suspension Test for the Evaluation of Sporicidal Activity of Chemical Disinfectants used in Food, Industrial, Domestic and Institutional areas - test method and requirements (phase 2, step 1)*

[32] European Standard EN 1276 *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)*

Household/commercial grade disinfectant wipes and sponges where the ingredient that is active cannot be expressed

Any reasonably designed test will be considered – a suggested method would include challenging the wipe or sponge by wiping it on a variety of surfaces. These surfaces should have been subject to periodic contamination with a microbiological bioburden. The wipe or sponge should be allowed to dry between uses. At the end of the use period, the product should be subjected to a simulated in-use test as described above. If claims against specific bacteria are made, then these organisms should be included in the test. Testing in accordance with EN 16615 will also be accepted.

Household/commercial grade disinfectants that are surface spray disinfectants

A household/commercial grade disinfectant that is a surface spray disinfectant is required to:

(1) Pass a bactericidal carrier test such as the AOAC Germicidal Spray Test. Testing in accordance with EN 14561 or EN 13697 will be accepted (test to be chosen depends on intended use). Sixty carriers per organism should be used, plus 10 carriers for each additional bactericidal claim. The performance criteria should be no growth from 59/60 carriers.

(2) Pass the tests specified in clause 1 with added inorganic soil, if intended as a surface spray disinfectant that is diluted before use. A surface spray disinfectant should be tested against *Salmonella enterica* serotype *choleraesuis* (*Salmonella choleraesuis*) *and Staphylococcus aureus* if the AOAC Germicidal Spray Test is used. If other tests are chosen, it is expected that organisms similar to these would be used.

(3) Pass carrier tests as described in previous clauses, if specific biocidal activities are claimed. Inorganic soil should be included.

Residual activity claims

1. If a claim of residual activity is made, the residual activity must be established using the methodology in:

(a) for bacteria or yeast—PAS 2424:2014 *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*, as in force or existing from time to time; or

(b) for bacteria—US EPA Protocol #01-1A *Protocol for Residual Self-Sanitising Activity of Dried Chemical Residues on Hard, Non-Porous Surfaces*, as in force or existing from time to time; or

(c) for viruses— ASTM E 1053[13] relating to a virucidal carrier test modified to include the abrasion and re-inoculation methodology in (a), or the wear and re-inoculation methodology in (b) would be considered. There is currently no standard method for a residual activity claim for a virus. Suitable virucidal efficacy test controls should be employed, including cytotoxicity controls, disinfectant neutralisation controls, quantitative viability control, cell control and carrier wash-off control. Guidance on carrier test methodology is provided in ASTM E 1053[13].

2. A claim of residual activity must be substantiated by test data that:

(a) meets acceptance criteria set at a 3-log difference between the test product and control product in their capability to continue to produce a reduction in the number of viable cells of relevant test organisms; and

(b) is derived using test methods justified with regards to the intended use of the disinfectant.

3. The period over which residual activity against bacteria, yeast or viruses is claimed must not be more than 30 days. For claims of a period longer than 24 hours, testing as per those

stipulated in methods a) PAS 242:2014 and b) US EPA Protocol #01-1A and c) ASTM E 1053 (modified) will be performed, with additional abrasion/wear and re-inoculation cycles over the period being claimed, including a final rechallenge step at the end of the period being claimed.

Note: Airborne disinfection technologies.

Airborne disinfection, often automated, is a widely used disinfection method in hospitals throughout the world, including Australia. Examples of such technologies include hydrogen peroxide vapour, hydrogen peroxide + peracetic acid fogging. Testing as described above may not be applicable to these types of technologies.

European standard EN 17272 - *Quantitative Carrier test for Airborne Room Disinfection by Automated Processes - Determination of Bactericidal, Fungicidal, Yeasticidal, Sporicidal, Tuberculocidal, Mycobactericidal, Virucidal and Phagocidal Activities in the Medical Area, Veterinary Area and Food, Industrial, Domestic and Institutional Areas - Test Methods and Requirements (Phase 2, Step 2)* is an appropriate test method. In addition, the French standard NFT 72 281 : 2014 *Methods of airborne disinfection of surfaces - determination of bactericidal, fungicidal, yeasticidal, mycobactericidal, tuberculocidal sporicidal and virucidal activity, including bacteriophages* is an acceptable test method.

Automated airborne disinfection systems also require in use validation using biological or chemical indicators, with 6-log *Geobacillus stearothermophilus* globally accepted as the microorganism to be used. The US EPA Protocol for Room Sterilization by Fogger Application (https://www.epa.gov/sites/production/files/2015-09/documents/room-sterilization.pdf) is an acceptable method for this testing.

Version history

Version	Description of change	Author	Effective date
V1.0	Original publication	Medical Devices Branch	March 2019
V2.0	Updated Table 1	Medical Devices Branch	October 2019
V2.1	Update to Virucidal testing	Medical Device Program	March 2020
V3.0	Update to residual activity claims, and minor clarifications	Medical Devices Authorisation Branch	December 2021

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