



Australian Government
Australian Pesticides and
Veterinary Medicines Authority



Guideline for data to support efficacy of disinfectants for veterinary use

AUGUST 2011

© Commonwealth of Australia 2011

This work is copyright. Apart from any use permitted under the *Copyright Act 1968*, no part may be reproduced without permission from the Australian Pesticides & Veterinary Medicines Authority. Requests and inquiries concerning reproduction and rights can be made to:

The Manager, Public Affairs
Australian Pesticides and Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604
Australia

Email: communications@apvma.gov.au

This document is published by the APVMA. In referencing this document the APVMA should be cited as both author and publisher.

Website: This publication is available from the APVMA website: <http://www.apvma.gov.au>

Comments and enquiries may be directed to:

John Owusu
Principal Evaluator, Veterinary Medicines Program
Australian Pesticides & Veterinary Medicines Authority
PO Box 6182
KINGSTON ACT 2604
Australia

Telephone: +61 2 6210 4730
Fax: +61 2 6210 4741
Email: john.owusu@apvma.gov.au

CONTENTS

ABBREVIATIONS	ii
<hr/>	
1. INTRODUCTION	1
1.1 Disinfectants for veterinary use	1
1.2. Classification of veterinary disinfectants and label efficacy claims	2
1.3. Efficacy claims against exotic diseases	2
2. EFFICACY CRITERIA	3
2.1. Test parameters for label claims for bactericidal, virucidal, fungicidal and sporicidal efficacy	3
3. GOOD MANUFACTURING PRACTICE (GMP)	7
APPENDIX 1: GLOSSARY OF TERMS	8
APPENDIX 2: EFFICACY TESTS	10
Section 1: Tests to determine bactericidal, virucidal, fungicidal and sporicidal efficacy	10
Section 2: The TGA Disinfectant Test to determine bactericidal efficacy	12
1 The principle of the TGA Disinfectant Test	12
2 Test conditions	12
3 Methodology	12
Section 3: Testing methodologies for assessing virucidal activity	17
1 The principle of the ASTM Virucidal Disinfectant Test	17
2. Test conditions and methodology	17
APPENDIX 3: GENERAL REQUIREMENTS FOR DISINFECTANT PRODUCT LABELS	18
1 Purpose and efficacy claims	18
2 Information required on the product label	18
3 Rinsing and waste disposal	18
4 Warning statements	19
5 Certain label statements may not be used	19
APPENDIX 4: GUIDELINES IN THE EN SERIES, AOAC SERIES AND ASTM SERIES THAT DESCRIBE EFFICACY TEST METHODOLOGIES FOR VETERINARY DISINFECTANTS	20
REFERENCES	23

ABBREVIATIONS

APVMA	Australian Pesticides and Veterinary Medicines Authority
AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
cfu	colony forming unit
EPA	Environmental Protection Agency
FAISD	First Aid Instruction and Safety Directions
g	grams
g/L	grams per litre
ID ₅₀	50% Infectious Dose
LD ₅₀	50% Lethal Dose
mL	millilitres
mm	millimetres
NATA	National Association of Testing Authorities
NCTC	National Collection of Type Cultures
OECD	Organisation for Economic Co-operation and Development
ppm	parts per million
SUSMP	Standard for the Uniform Scheduling of Medicines and Poisons
TCID ₅₀	50% Tissue Culture Infective Dose
TGA	Therapeutic Goods Administration
Vet MORAG	Veterinary Manual of Requirements and Guidelines

1. INTRODUCTION

Applicants seeking registration of a disinfectant product for veterinary use must submit efficacy and safety data to support all label claims. The objective of this guideline is to provide advice on the data requirements for the registration of disinfectants for veterinary use with generic label claims. Data requirements for the registration of disinfectants for veterinary use with specific claims will be considered on a case by case basis. Irrespective of whether efficacy claims for a veterinary disinfectant are generic or specific, applicants are encouraged to seek advice from the Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluators before submitting an application.

In addition to efficacy and safety, a disinfectant for veterinary use must satisfy the APVMA's criteria for chemistry and manufacture, public health, environmental chemistry and fate, and occupational health and safety. For this reason, the requirements and guidance in this document should be read in conjunction with:

- the relevant sections of other applicable guidance documents set out in the Veterinary Manual of Requirements and Guidelines (Vet MORAG) (ref 1)
- the Vet Labelling Code (ref 2)
- the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP) (ref 3)
- First Aid Instruction and Safety Directions (FAISD) Handbook (ref 4).

This guideline includes four Appendices. Appendix 1 is a glossary of terms relating to the efficacy of veterinary disinfectants. Appendix 2 describes the testing methodologies that APVMA has endorsed as being suitable for demonstrating efficacy in support of label claims. It also provides additional information on efficacy tests for veterinary disinfectants. Appendix 3 describes particular requirements for disinfectant product labels, which, as mentioned above, must be read in conjunction with the Vet Labelling Code (ref 2), SUSMP (ref 3) and FAISD Handbook (ref 4). Appendix 4 provides guidance as to other tests that are available. It tabulates those guidelines in the EN series (European), AOAC series (USA) and ASTM series (International) that describe efficacy test methodologies for veterinary disinfectants. Also presented in Appendix 4 are the applications (or indications) for each of the tests listed and information on the test conditions.

1.1 Disinfectants for veterinary use

For the purpose of this guideline, veterinary disinfectants are products that are used on hard, inanimate objects or surfaces to destroy a range of microorganisms. Two general categories of veterinary disinfectants are recognised by the APVMA for registration purposes and are based on the situations in which the disinfectants are used. These are:

- areas that generally have a lower level of contamination such as waiting rooms, consulting rooms and surgical suites in veterinary clinics/hospitals.
- areas that generally have a higher level of contamination such as preparation areas and animal cages/runs within a veterinary clinic or hospital, and animal housing facilities such as dog kennels, catteries, horse stables, poultry houses, cattle and sheep facilities, and piggeries. This category of veterinary disinfectants may also be suitable for use on animal transport vehicles.

A third albeit less common category of disinfectants for veterinary use comprises those with specific label claims e.g. virucidal activity only. This category of veterinary disinfectant is considered on a case by case basis by the APVMA for registration.

For the purpose of this guideline, veterinary disinfectants do not include products that are:

- used on surfaces which come into contact with food (Sanitisers)
- used on animals, either externally or internally (Antiseptics).

1.2. Classification of veterinary disinfectants and label efficacy claims

The label claims for veterinary disinfectants must reflect the fitness-for-purpose of the product, regardless of where the product is used within a veterinary context. The minimum requirement for registration purposes is demonstration of efficacy against the infectious agents described as mandatory in this guideline.

Demonstrable efficacy against these organisms will provide for a generic claim on the product label.

Applicants may elect to demonstrate efficacy against additional infectious agents and in this respect, the product label may list a generic claim and one or more specific claim(s) that have been substantiated by data.

Veterinary disinfectant products for use in veterinary clinics/hospitals must be suitable for disinfecting facilities such as waiting rooms, consulting rooms, surgical suites, and for disinfecting surgical instruments. The latter requires that the product is non-corrosive.

Veterinary disinfectant products for use in animal housing facilities as well as preparation areas and animal cages/runs within veterinary clinics/hospitals must satisfy the efficacy criteria as per Table 1.

1.3. Efficacy claims against exotic diseases

In February 2008, the APVMA gazetted an operational notice stating that disinfectant labels may not make claims for efficacy against diseases that are exotic to Australia (ref 5). Efficacy claims should be for endemic diseases only.

The label disclaimer should note that the disinfectant product does NOT protect against exotic diseases.

Exotic diseases are dealt with separately on a case by case basis and this may require the issuance of an emergency permit following the incursion of an exotic disease.

2. EFFICACY CRITERIA

Before registering a veterinary disinfectant product, the APVMA evaluates data derived from efficacy studies relevant to the claims being made.

Efficacy data in support of the registration of a veterinary disinfectant must be technically sound, reproducible and reliable. To ensure that this is the case, efficacy tests must be conducted by laboratories that are accredited to conduct the tests by the National Association of Testing Authorities (NATA) or an equivalent accreditation body. Efficacy tests conducted by these laboratories comply with the OECD Principles of Good Laboratory Practice (GLP).

The APVMA has endorsed the tests shown in Appendix 2 but will also consider, on a case by case basis, efficacy data that have been generated using other tests, including internationally accepted test standards and new technologies. Appendix 4 provides guidance as to other tests that are available. When tests other than those shown in Appendix 2 are used, the applicant will be required to submit scientific justification to support such data. The APVMA will then determine whether the methods used are equivalent to those described in Appendix 2 in this guideline.

Applicants requiring advice on appropriate data for specific product applications are encouraged to contact relevant evaluation staff in the Veterinary Medicines Program of the APVMA.

2.1. Test parameters for label claims for bactericidal, virucidal, fungicidal and sporicidal efficacy

Applicants must demonstrate the efficacy of the proposed product by using acceptable test methods to support label claims. Appropriate methods should address:

- fitness-for-purpose of the product – the method protocol should reflect the intended use pattern of the product and this dictates the test parameters to be investigated e.g. growth on a solid surface or in a liquid suspension, contact times, microbiological species and strains. In this respect, some flexibility regarding the substrate used for test micro-organism is acceptable as shown in Appendix 4.
- interference/confounders – efficacy validated in the presence of known interferences such as soiled environments (see also Section 2.1.1), hard water
- controls/validation – adequate controls to validate the protocol which include stopping disinfectant action, cytotoxicity, and other controls as listed in this guideline (see also Sections 2.1.1 and 2.1.5)

Additional information on those efficacy tests preferred by the APVMA is described in Appendix 2. Included is a detailed description of methodology based on the TGA Disinfectant Test (ref 6) with modifications to suit veterinary situations. Appendix 2 also provides additional detail on the testing methodologies for assessing virucidal activity.

If the applicant decides to use a test methodology other than those described in Appendix 2, proposals will need to be submitted to the APVMA for consideration. In these cases, the following points should be observed:

1. High-level soiling conditions should be used when it is necessary to simulate the conditions of animal housing. If the methodology does not provide specific instruction about interfering substances applicable to the veterinary field, the test conditions described in Section 2.1.1 should be used.

2. The performance standards described in Section 2.1.2 should be used when values are not specified in the description of the test methodology.
3. The organisms described in Section 2.1.3 should be used with all test protocols (bactericidal, fungicidal, sporicidal or virucidal).
4. The validation steps described in the chosen method as well as those described in Section 2.1.5 should be performed.

2.1.1. Test conditions

The applicant must describe the test conditions as well as the positive and negative controls used in the efficacy tests performed.

Efficacy tests for disinfectants must be conducted:

- to simulate a contaminated environment which mimics preparation areas and animal cages/runs within veterinary clinics/hospitals and animal housing, the test must be performed in the presence of organic and inorganic matter such as killed yeast, serum, or serum albumin in order to simulate *in vitro* contaminated conditions. In the absence of a specification for contamination, a mixture of serum and killed yeast should be used such that the final test concentrations are 0.5% w/v killed yeast and 5% v/v sheep serum.

All efficacy tests must be conducted:

- using hard water¹ (342 ppm calculated as calcium carbonate (80:20 Ca⁺²:Mg⁺²)) as a diluent for those products that are diluted before use. A method for preparing sterile hard water is described in Appendix 2
- at the simulated in-use conditions in respect of pH², temperature³, exposure time-concentration (dilution) combinations, single or repeated applications, and surfaces should be identical to the directions for use on the product label
- immediately prior to the end of the proposed shelf-life of the product.

2.1.2. Performance Standards

The performance standards described in this section apply only if the applicant chooses to use a test method that differs from the preferred efficacy tests described in Appendix 2. Test protocols must be described in detail to facilitate a valid assessment of the data.

For bacterial testing methodologies not included in Table 2, the product must be challenged with a minimum of 10⁸ cfu. The acceptance criterion for passing the test is a 10⁶-fold reduction in microorganisms (other than viruses) in a suspension or on an inanimate surface at a specified contact time and temperature.

Viruses should be tested in the appropriate cell culture and using appropriate controls (refer to Appendices 2 and 4) and neutralisation of the disinfectant under test (refer to Section 2.1.5). The cell cultures and growth conditions used in these studies must be validated. For the product to pass the test, a minimum of a 10⁴-fold reduction in virus titre must be demonstrated from an initial inoculum of approximately 10⁸ TCID₅₀. When cytotoxicity is evident, a 10³-fold reduction, or greater, beyond the cytotoxic level must be demonstrated. This

¹ The degree of hardness of water (i.e. the presence of Ca⁺² and Mg⁺²) used to dilute the disinfectant may affect its performance. Generally the harder the water the less effective is the diluted disinfectant.

² The prevailing degree of acidity or alkalinity during disinfection can affect the performance of a disinfectant. Generally, disinfectants are more active as undissociated molecules than as ionised molecules.

³ Generally disinfection performance increases with temperature. This applies to disinfection against all microorganisms though the effect on individual species differs, some being more affected than others.

is in accordance with the cytotoxicity testing protocol of the US Environmental Protection Agency (ref 7). Testing methodologies to assess virucidal activity are described in Appendices 2 and 4.

2.1.3. Test organisms

Test organisms should be selected from a recognised culture collection and their phenotypic/genotypic characteristics must be known and checked regularly. Examples of recognised culture collections include the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC). If the test organisms have not been classified at a reference centre, detailed characterisation of the test organisms must be submitted.

Table 1 lists the test organisms for disinfectant products for use in various situations. Table 1 must be read across the matrix, not down. Apart from exceptional circumstances, the APVMA will not register, based on the organisms shown in Table 1, a veterinary disinfectant with bactericidal, virucidal, fungicidal, or sporicidal activity only. However, claims for these individual activities are possible provided specific claims of efficacy are substantiated by efficacy data.

The selection of test organisms for efficacy testing should be relevant to the intended use and claim of the veterinary disinfectant product. A label claim for use of a disinfectant in any veterinary clinic/hospital and all categories of animal housing (cats, dogs, horses, cattle, sheep, pigs and poultry) requires, as a minimum, that efficacy is demonstrated against all organisms listed in Row A of Table 1. A label claim for an individual category of animal housing requires that efficacy is demonstrated against the microorganisms in Table 1 for the particular animal species. For example a claim for 'Dog kennel, cattery or piggery only' requires demonstration of efficacy against organism in Row B. The test protocols are designed to simulate an environment mimicking the level of contamination in preparation areas and animal cages/runs in veterinary clinics/hospitals and animal housing facilities.

The test organisms shown in Table 1 have been selected on the basis that they are more resistant than other microorganisms to the effect of disinfectants. Demonstrable efficacy to these test organisms will be accepted as evidence to support a generic claim in those situations described in Table 1. For example, disinfectants that demonstrate efficacy against canine parvovirus, the test virus in Table 1, will be considered an effective virucidal for use in Australian veterinary clinics/hospitals while the use of a human rotavirus (rotavirus Wa strain) is recommended as a surrogate test virus for veterinary rotaviruses. Other examples include vaccinia WR strain which is recommended as a surrogate test virus for veterinary poxviruses, and canine parvovirus which is recommended as a surrogate test virus for feline and porcine parvoviruses. This reflects the availability of standard protocols for these surrogate test viruses and the relative ease with which these surrogate test viruses can be grown and titrated in the laboratory. Other viruses within the specified virus families (rotaviruses, poxviruses, parvoviruses) may also be suitable for use as test viruses; however, their use would need to be justified scientifically.

Table 1 Test organisms for veterinary disinfectant products for use in veterinary clinics/hospitals and all categories of animal housing* or individual categories of animal housing facilities**

	Situations	TEST ORGANISMS			
		Bacterium	Virus	Fungus	Spore-forming
A	Veterinary clinics/hospitals & all categories of animal housing	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Canine parvovirus Cornell strain	<i>Trichophyton mentagrophytes</i>	Spores of <i>Bacillus subtilis</i>
B	Dog kennel, cattery or piggery only	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Canine parvovirus Cornell strain	<i>Trichophyton mentagrophytes</i>	---
C	Horse stable only	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Rotavirus Wa strain, Vaccinia WR strain ¹	<i>Trichophyton mentagrophytes</i>	Spores of <i>Bacillus subtilis</i>
D	Poultry house only	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Canine parvovirus Cornell strain ²	<i>Aspergillus niger</i>	Spores of <i>Bacillus subtilis</i>
E	Cattle/sheep facility only	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella typhimurium</i> , <i>Escherichia coli</i>	Rotavirus Wa strain, Vaccinia WR strain ¹	---	Spores of <i>Bacillus subtilis</i>

* Disinfectants for use in veterinary clinics/hospitals must be non-corrosive.

** A disinfectant product for use in animal housing may be approved for use in animal housing for one or more animal species.

¹ Two test viruses (a poxvirus and a rotavirus) are recommended in horse stables and cattle/sheep facilities. This is necessary as poxviruses can demonstrate a higher level of resistance to some types of disinfectants whereas rotaviruses can demonstrate a higher level of resistance to other disinfectant types.

² The recommendation to use canine parvovirus as a test virus to demonstrate virucidal activity in poultry houses takes into account the difficulties associated with using chicken anaemia virus and other circoviruses as test viruses. Applicants are, however, still able to submit, for consideration, a proposed protocol to assess virucidal activity against circoviruses.

2.1.4. Number of batches to be tested

Applicants must submit the results of efficacy tests in triplicate from at least three separate batches. All samples must pass the efficacy tests in order for the results to be accepted.

2.1.5. Validation of test methods

Efficacy test methods must be validated and use appropriate controls and reference organisms. Where reference materials are specified in a test method, they must be used at the nominated concentration. It may be necessary to validate test methods using a reference substance such as:

- benzalkonium chloride (the chain length and concentration as per the US Pharmacopeia) for

bactericidal tests

- 0.05 per cent phenol (see AOAC 955.11 for reference; ref 8) for bactericidal and fungicidal tests
- 2 per cent glutaraldehyde for sporicidal tests
- hypochlorite for virucidal tests

Some test methods include a neutralisation step to stop the action of the disinfectant at a specified time. A neutralisation step is mandatory when validating these test methods. Other test methods (e.g. ASTM E 2197-02; ref 9) include a filtration/washing step after contact of the infectious agent with the disinfectant. A neutralisation study is optional with these test methods.

When demonstrating effective neutralisation of the disinfectant under test, the applicant must conduct a study in which a neutralising agent is added to the samples of both treated and untreated control groups. The neutralising agent must completely inhibit the activity of the disinfectant without being toxic to the indicator organisms. In addition, the neutralising agent and active constituent must not combine to form a toxic compound. Some examples of neutralising agents are 3 grams per litre (g/L) lecithin, 30 g/L polysorbate 80, and 5 g/L sodium thiosulphate. The applicant may wish to refer to ASTM E 1054–08 (ref 10), which describes a neutraliser validation test.

3. GOOD MANUFACTURING PRACTICE (GMP)

Disinfectants are defined in Section 4 of the Agvet Code as agricultural chemical products used in a veterinary situation (ref 11). As such, they are exempt from the requirement of the Agvet Code to be manufactured in premises licensed by the APVMA under the Australian Code of Good Manufacturing Practice for Veterinary Chemical Products.

APPENDIX 1: GLOSSARY OF TERMS

Antiseptic	An antiseptic is a substance that is recommended by its manufacturer for dermal application or application to the mucous membranes of an animal to kill microorganisms, or to restrict the growth of microorganisms to below a level that may cause clinical infection, and that is not represented to be suitable for internal use.
Bactericide	A product which kills vegetative bacteria under defined conditions
Bactericidal activity	The capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions
Biofilm	An accumulation of microbial cells immobilised on a substratum and embedded in an organic polymer matrix of microbial origin
Fungicide	A product which kills fungi (vegetative mycelia, budding yeasts and/or their spores) under defined conditions
Fungicidal activity	The capability of a product to produce a reduction in the number of viable vegetative yeast cells and mould spores of relevant test organisms under defined conditions
Hard water	Water containing 342 ppm calculated as calcium carbonate (80:20 Ca ⁺² :Mg ⁺²)
ID ₅₀	The 50% infectious dose (ID ₅₀) is the virus dilution where 50% of the inoculated hosts are infected
Infectious unit	The smallest amount of virus that produces some recognisable effect in the host system employed
LD ₅₀	The lethal dose that kills 50% of the inoculated animals
Neutraliser	A chemical agent or formulation which suppresses the residual activity of a disinfectant within a test but does not inhibit or inactivate microorganisms
Performance standard	A regulatory or scientific standard for disinfectants that is either quantitative or qualitative (that may also be specified in the test method) by which a decision is taken on the acceptability of a claim
Sanitiser	A chemical agent that is represented to be suitable for use in the reduction of pathogenic or food spoilage microorganisms to a sanitary level on surfaces with which food for human consumption may come in contact. [Note that pool sanitisers are distinct agricultural chemical products].
Soiling/contamination	The presence of organic matter in the environment where animals are cared for or housed that is attributable to the presence of animals and harbours microorganisms
Sporicide	A product which kills dormant bacterial spores under defined conditions
Sporicidal activity	The capability of a product to produce a reduction in the number of viable bacterial spores of relevant test organisms under defined conditions
TCID ₅₀	The 50% tissue culture infective dose (TCID ₅₀) is that dilution of virus required to infect 50% of the cell cultures inoculated
Titre	A given number of infectious virus units per unit volume
Virucide	A product which inactivates virus under defined conditions

Virucidal activity	The capability of a product to produce a reduction in the number of infectious virus particles of relevant test organisms under defined conditions
--------------------	--

APPENDIX 2: EFFICACY TESTS

The data requirements described in this guideline apply to veterinary disinfectants with bactericidal, virucidal, fungicidal and sporicidal activities. Although Section 2 refers solely to bactericidal activity and Section 3 refers solely to virucidal activity, these Sections are not intended to describe the data requirements for a veterinary disinfectant demonstrating bactericidal activity only, or virucidal activity only. Rather they provide additional detail on the data requirements relating to microbicidal activities for a veterinary disinfectant that demonstrates efficacy against bacteria, viruses, fungi and spores.

The APVMA has endorsed the tests described in this Appendix as being suitable for demonstrating efficacy in support of label claims. Efficacy claims against specific microorganisms must be supported by data generated using the test organisms and parameters described in Section 2.1 of this guideline.

The test protocols described in this guideline do not assess the efficacy of disinfectants against microbes associated with biofilms. The latter are formed when bacteria and fungi adhere to surfaces and their cell surface properties are altered. This can result in reduced penetration and efficacy of disinfectants against the microbes.

The infectious agents in Table 1 have been selected for their resistance to veterinary disinfectants and the practicalities of being able to grow them in the laboratory. The testing of these microorganisms overcomes the need to test infectious agents that are less resistant to disinfectants. For example, if the product is demonstrably efficacious against canine parvovirus, then a label claim against all canine viruses will be granted.

Section 1: Tests to determine bactericidal, virucidal, fungicidal and sporicidal efficacy

The purpose of this section is to summarise the main test methodologies that are available for evaluating the bactericidal, virucidal, fungicidal and sporicidal efficacy of veterinary disinfectants. Applicants should use the test protocols described in Table 2 in combination with the test organisms shown in Table 1 of this guideline. The label claim of the product will determine the appropriate test organisms from Table 1. Claims for efficacy against infectious agents in addition to the mandatory test organisms shown in Table 1 will be considered on the basis of demonstrable efficacy.

Table 2: Tests for bactericidal, virucidal, fungicidal or sporicidal claims of disinfectants¹

CLAIM	TEST METHODS	ORGANISM USED
Bactericidal, Virucidal, Fungicidal, or Sporicidal	ASTM E 2197-02, Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides (ref 9) Use soiling conditions as recommended	Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196), canine parvovirus Cornell strain (ATCC VR-2017), Rotavirus Wa strain (ATCC VR-2018), Vaccinia WR strain (ATCC VR-119), Trichophyton mentagrophytes (ATCC 9533), Aspergillus niger (ATCC 16404), Bacillus subtilis (ATCC 19659)
Bactericidal	1. The efficacy testing requirements described in 'TGA Disinfectant Test' for Option B (ref: Section 2 of this Appendix) OR 2. EN 1656, Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field –	Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196) Staphylococcus aureus (ATCC 6538), Pseudomonas aeruginosa (ATCC 15442), Salmonella typhimurium (ATCC 10708), Escherichia coli (NCTC 8196)

	Test method and requirements (phase 2, step 1) (ref 12)	
	Use high level soiling (10g/L bovine albumin and 10g/L yeast extract) as recommended	
Virucidal	1. ASTM E 1053-97 – Standard Test Method of Virucidal Agents Intended for Inanimate Environmental Surfaces (ref 13) Use soiling conditions as recommended	Canine parvovirus Cornell strain (ATCC VR-2017), Rotavirus Wa strain (ATCC VR-2018), Vaccina WR strain (ATCC VR-119)
	OR	
	2. EN 14675, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area — Test method and requirements (phase 2, step 1) (ref 14). Use high level soiling (10g/L bovine albumin and 10g/L yeast extract) as recommended	Canine parvovirus Cornell strain (ATCC VR-2017), Rotavirus Wa strain (ATCC VR-2018), Vaccina WR strain (ATCC VR-119)
Fungicidal	EN 1657, Chemical disinfectants and antiseptics—Quantitative Suspension Test for the evaluation of fungicidal or yeasticidal activity chemical disinfectants and antiseptics used in the veterinary area – Test method and requirements (phase 2, step 1) (ref 15) Use high level soiling (10g/L bovine albumin and 10g/L yeast extract) as recommended	Trichophyton mentagrophytes (ATCC 9533), Aspergillus niger (ATCC 16404)
Sporicidal	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) (ref 16). Use a high level soiling (10g/L bovine albumin and 10g/L yeast extract)	Spores of Bacillus subtilis (ATCC 19659 or ATCC 6633)

¹ Test conditions are described in Section 2.1.1.

Section 2: The TGA Disinfectant Test to determine bactericidal efficacy

The purpose of this section is to describe the test methods for determining bactericidal efficacy for the two general categories of veterinary disinfectants, namely disinfectants for use in waiting rooms, consulting rooms and surgical suites in veterinary clinics/hospitals, and disinfectants for use in the preparation areas and animal cages/runs in veterinary clinics/hospitals and in animal housing facilities. The test conditions mimic worse case environments. The methodology described below is based on the TGA Disinfectant Test with modifications to suit veterinary situations.

1 The principle of the TGA Disinfectant Test

The TGA Disinfectant Test (ref 6), also known as a Kelsey-Sykes Capacity In Use Test (ref 17), assesses the capacity of the disinfectant to destroy successive additions of a bacterial culture. The conditions of this test are an attempt to reproduce some of the conditions that disinfectants must overcome in normal use.

The disinfectant is tested at the dilution recommended on the product label. The test consists of challenging the diluted disinfectant with bacterial inoculum, withdrawing a sample after a given time and culturing the sample in a suitable recovery medium. After this sampling, the mixture is again challenged by a second inoculum and after a second interval is again sampled for culturing. The sample is passed or failed according to the extent of growth in the cultures sampled.

2 Test conditions

The bactericidal efficacy tests for veterinary disinfectants must be conducted using the organisms and conditions in Table 3. In all cases, sterile hard water must be used as the diluent. The test conditions are simulated by the addition of organic matter in the form of a mixture of killed yeast suspension and sheep serum.

Table 3 shows the test parameters to demonstrate a general bactericidal effect for veterinary disinfectants.

Table 3: Selection of test parameters for veterinary disinfectants

TEST ORGANISMS	TEST OPTION FOR RE-SUSPENSION OF CENTRIFUGED ORGANISMS	NUMBER OF CHALLENGES	INOCULUM DENSITY
1. <i>Staphylococcus aureus</i>	TGA Option B combination of killed yeast suspension and sheep serum and using hard water as the diluent	2	1 x 10 ⁸ to 1 x 10 ⁹
2. <i>Salmonella typhimurium</i>			
3. <i>Pseudomonas aeruginosa</i>			
4. <i>Escherichia coli</i>			

Testing of the disinfectant must use all four organisms separately.

3 Methodology

Media

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

Sterile hard water

Dissolve 0.304 grams (g) anhydrous calcium chloride and 0.065 g anhydrous magnesium chloride in glass-distilled water, and make up to one litre. The final concentration is 2.7 mM CaCl₂, 0.7 mM MgCl₂.

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

Killed yeast suspension to simulate 'contaminated' conditions

- 1 Weigh 200 g 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 500 millilitres (mL) of water has been used.
- 2 Shake the contents of the flask vigorously and strain through a 100-mesh sieve, breaking down any remaining lumps.
- 3 Add 500 mL sterile hard water, shake vigorously and adjust the pH to 6.9–7.1 with 1N sodium hydroxide.
- 4 Transfer 50 mL, 100 mL or 200 mL of the yeast solution into screw-capped bottles.
- 5 Autoclave at 121 ± 1 °C for 15 minutes and allow the autoclave to cool without releasing pressure. Store at 4–8 °C.
- 6 Dry two Petri dishes to constant weight. Into each, pipette 25 mL of sterilised yeast suspension, and dry to constant weight at 100 °C. Calculate the average solids content of the suspension.
- 7 Before use, pipette 25 mL 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.
- 8 Immediately before use, add to each bottle of sterilised yeast, a volume of sterile hard water to adjust the concentration of dry yeast to 5.0%, and a pre-determined volume of 1N NaOH to adjust the pH to within the range 6.9–7.1. Discard prepared yeast 3 months after preparation.

Medium for growth of test organisms

- 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 Prepare Wright and Mundy medium following the author's procedure (ref 18) or from a commercial product of the same composition (see Supplementary Note A), and sterilise by autoclaving at 121 ± 1 °C for 15 minutes. Cool to room temperature.
- 3 To each litre of Wright and Mundy medium prepared in step 2, add 10 mL sterile dextrose solution.
- 4 Aseptically dispense in either 10 mL or 15 mL amounts, as preferred. This medium is referred to as Wright and Mundy dextrose medium.

Recovery medium

- 1 Prepare nutrient broth as follows or from a commercial product of the same composition (see Supplementary Note A):
Add the following to 970 mL of water and dissolve by heating.
Beef extract powder 10 g
Peptone 10 g
Sodium chloride 5 g
Adjust the pH to 8.0–8.4 using 1N sodium hydroxide.
Boil for 10 minutes and filter. Cool.
- 2 To each litre of nutrient broth solution prepared in step 1, add 30 g polysorbate 80 (see Supplementary Note B).
- 3 Adjust pH to 7.2–7.4, using 1N sodium hydroxide.
- 4 Autoclave at 121 ± 1 °C for 15 minutes and immediately shake well to disperse the polysorbate 80.
- 5 Dispense aseptically in 10 mL amounts into sterile capped glass tubes.

Test inoculum

Test organisms

- *Staphylococcus aureus* (ATCC 6538 or NCTC 4163)
- *Salmonella typhimurium* (ATCC 13311)
- *Pseudomonas aeruginosa* (ATCC 15442 or NCTC 6749)
- *Escherichia coli* (NCTC 8196)

Preparation of inoculum

- 1 Incubate the contents of an ampoule of freeze-dried culture overnight at 37 ± 1 °C in Wright and Mundy dextrose medium.
- 2 Inoculate the incubated culture onto nutrient agar slopes in McCartney bottles. Store for up to 3 months at 4 ± 1 °C.
- 3 At a suitable period before the test is to be conducted, sub-culture from an agar slope into 10 mL or 15 mL quantities of Wright and Mundy dextrose medium. Incubate at 37 ± 1 °C for 24 ± 2 hours.
- 4 Sub-culture from the medium in step 3 into fresh medium, using an inoculating loop of 4 mm diameter. Incubate at 37 ± 1 °C for 24 ± 2 hours.
- 5 Repeat step 4 daily. For the test procedure use only those cultures which have been sub-cultured at least 5, and not more than 14, times.
- 6 Filter test cultures of *P. aeruginosa* and *S. aureus* through sterile Whatmans No. 4 filter paper to remove cell debris and clumps. Use the flow through as the test culture suspension.
- 7 Centrifuge all test cultures until cells are compact, and remove supernatant with a Pasteur pipette.
- 8 Re-suspend test organisms in the original volume of liquid (i.e. 10 mL or 15 mL sterile hard water containing 2% w/v killed yeast and 20% v/v sheep serum), and shake for 1 minute with a few sterile glass beads.

Enumeration of inoculum

Immediately before testing, take an aliquot of the re-suspended inoculum and prepare a series of 10-fold dilutions in a suitable diluent such as quarter-strength Ringer's solution. Inoculate 1 mL from the dilution 10^{-6} , 10^{-7} and 10^{-8} in duplicate using the pour-plate technique. Retain the tube containing 10^{-7} dilution for use as a control (see Fertility and Inactivator Efficacy tests below). Incubate the plates overnight and calculate the titre

of the suspension. The number subsequently counted must represent not less than 10^8 or more than 10^9 cfu/mL or the test is considered invalid.

Disinfectant dilutions

Dilute a sample of the disinfectant to the specified extent, using sterile hard water as diluent. Use not less than 10 mL or 10 g of sample for the first dilution, and not less than 1 mL 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.

Temperature

Where air-conditioning does not maintain test solutions at $21 \pm 1^\circ\text{C}$, hold the containers in which the test is to be carried out in a water bath at this temperature.

Test procedure

Perform the following test using each of the four test organisms listed under 'Test organisms'. It is not necessary to test with all organisms simultaneously.

- 1 Add 3 mL of diluted disinfectant to a capped glass container.
- 2 Start a timing device. Immediately inoculate disinfectant with 1 mL of re-suspension (see 'Preparation of inoculum', point 8) and mix by swirling.
- 3 At 8 minutes, subculture one drop ($0.02 \pm .002$ mL) into each of 5 tubes containing recovery broth. To ensure delivery of 0.02 mL 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. (See supplementary Note B.)
- 4 At 10 minutes, inoculate disinfectant with a further 1 mL of culture, and mix by vortexing.
- 5 At 18 minutes, proceed as in step 3.
- 6 Mix the contents of all tubes of recovery broth by vortexing. Incubate at $37 \pm 1^\circ\text{C}$ for 48 ± 2 hours.
- 7 Examine for growth and record results.
- 8 For each test organism, repeat all the above steps on each of 2 subsequent days using a fresh disinfectant dilution and a freshly prepared bacterial suspension.

Controls

Recovery broth contamination

Incubate one un-inoculated tube of recovery broth at $37 \pm 1^\circ\text{C}$ for 48 ± 2 hours and examine for growth. If growth occurs, the test is considered invalid due to contamination of the recovery broth.

Disinfectant contamination

To 1 tube of recovery broth, add 0.02 mL of diluted disinfectant. Incubate at $37 \pm 1^\circ\text{C}$ for 48 ± 2 hours. If growth occurs, the test is considered invalid. Growth in this step, but not in the above step, indicates contamination of the disinfectant test solution.

Fertility test

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

Inactivator efficacy

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

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 the control 'recovery broth contamination' or if no growth occurred in the control 'fertility test' and 'inactivator efficacy'.

Should disinfectant contamination be indicated on both occasions, the disinfectant is considered to have failed the test.

Should inadequate inactivation of the disinfectant be indicated by control 'inactivator efficacy' on both occasions, the test is considered invalid (see supplementary Note C).

Results

The dilution test passes if there is no apparent growth in at least two of the five recovery broths specified in the test procedure, on all three occasions and using all four organisms. The test fails if the test for one of the four organisms is failed.

Supplementary notes

- [A] Wright and Mundy medium is commercially available as 'Bacto Synthetic Broth', AOAC Code No. 0352 (Difco Ltd.). The nutrient broth to be used is available as 'Nutrient Broth - No. 2' (Oxoid Ltd.).
- [B] The Oxford P-7000 sampler system with disposable plastic tips is recommended for the withdrawal of samples for sub-culturing.
- [C] Where inadequate inactivation is indicated, investigations should be conducted to find an effective inactivator (ref 19).

Acknowledgement

This method has been reproduced with the permission of the TGA and includes some modification.

Section 3: Testing methodologies for assessing virucidal activity

The purpose of this section is to elaborate on the testing methodologies for assessing virucidal activity, as described in ASTM E 1053–97 (ref 13).

1 The principle of the ASTM Virucidal Disinfectant Test

The laboratory test method described in ASTM E 1053-97 (ref 13) is designed to evaluate the virucidal efficacy of disinfectants on inanimate surfaces. This protocol uses the surface of glass petri plates to represent environmental surfaces. An alternative protocol utilising small carrier disks of stainless steel is described in ASTM International Designation E 2197–02 (ref 9). Both protocols are suitable for use with any of the recommended test viruses described in Table 1.

Briefly, in ASTM E 1053-97 (ref 13), a high titre of virus suspension is prepared (10^7 - 10^8 infectious units/mL) and the virus suspension is dried on the test surface. The disinfectant is then applied to the dried film for the recommended time, as indicated on the instructions for use on the label of the disinfectant. The virus-disinfectant mixture is then resuspended and the virus titre is determined in cell culture. The extent of virus inactivation by the antimicrobial agent is determined and is recorded as the \log_{10} reduction in viral titre. The virus-disinfectant mixture is assayed at a dilution just beyond the cytotoxicity range of the disinfectant, as determined by an LD_{50} method to assess cytotoxicity. In addition to this virucidal test, the protocol also requires control parameters in the form of cell culture control, virus control, cytotoxicity control and neutralisation control.

2. Test conditions and methodology

The applicant is referred to ASTM E 1053–97 (ref 13), or alternatively E 2197-02 (ref 9), for detailed testing methodology and testing conditions for canine parvovirus, human rotavirus and vaccinia virus.

These ASTM protocols do not specify the requirements for passing the virucidal test. Therefore, this Guideline applies the requirements of the US Environmental Protection Agency (i.e. a 10^4 -fold reduction in titre, or a 10^3 -fold reduction in viral titre beyond any disinfectant dilutions which exhibit cell culture cytotoxicity) for passing the virucidal test (ref 7).

APPENDIX 3: GENERAL REQUIREMENTS FOR DISINFECTANT PRODUCT LABELS

1 Purpose and efficacy claims

There should be no ambiguity regarding each of the intended uses of the product. The product label must clearly identify the purpose of the product (for example, use in veterinary hospitals, use in dog kennels, use in horse stables etc) and the scope of the efficacy being claimed, so that the user will clearly understand the product's intended use. The latter must align with the general categories defined in Section 1.1. The lowest use-dilution recommended on the label must induce a microbiocidal effect at the log reduction of the test organism specified in this guideline.

2 Information required on the product label

The label must specify the instructions for use including the following:

- use pattern
- site and method of application
- area and type of surfaces (eg steel, porcelain, aluminium, glass, chrome, vinyl, rubber, plastic, polymeric flooring) on which the veterinary disinfectant may be applied
- contact time
- temperature
- pH
- concentration
- mixing directions
- mode of application and dose rates for the intended use.

The label must inform the user for how long a diluted and/or mixed disinfectant solution may be kept, reused and stored.

The label must indicate that the disinfectant should not be used on surfaces which come into contact with food for human consumption.

The label must indicate that no animal should come into contact with the disinfectant. Animals, feed, water, bedding, litter and fomites should be removed from the area during the treatment. The label must clearly specify pre-cleaning requirements of surfaces to remove gross contamination, prior to use of the disinfectant product.

The label must indicate that the efficacy of the disinfectant against microbes associated with biofilms has not been assessed.

3 Rinsing and waste disposal

The label must provide instructions when a rinsing step is required to remove the disinfectant from a surface to which it has been applied. Animals, feed, water, bedding, litter and fomites should only be reintroduced to animal housing, and the use of feeding and watering appliances should only be recommenced, after the surface with applied disinfectant product has been thoroughly cleaned with soap or detergent to remove residues and allowed to dry.

The label must provide a waste disposal statement for those disinfectants intended to be used in large volumes.

4 Warning statements

The label must provide safety instructions for personnel and target animals including any necessary precautionary measures.

5 Certain label statements may not be used

Non-specific label statements such as 'non-corrosive', 'non-toxic', 'non-irritant', 'safe', 'non-caustic', 'harmless' must not be used for disinfectant products, unless supported by appropriate data.

APPENDIX 4: GUIDELINES IN THE EN SERIES, AOAC SERIES AND ASTM SERIES THAT DESCRIBE EFFICACY TEST METHODOLOGIES FOR VETERINARY DISINFECTANTS

Application	Interfering substances	Substrate used for test microorganism	Species of microorganism	Titre of microorganism	Temperature and time	Performance standard	Additional information	Reference
Bactericidal	5% v/v serum option in additional guidance	Surface carrier (stainless steel)	<i>Staphylococcus aureus</i> (ATCC 6538),	1×10^6 ; a 48 h-54 h liquid culture in broth is used	20°C, 10 min	Liquid culture of carriers: less than 1 positive out of 60 tested	<u>Controls:</u> Viability controls, verification of positive carriers, neutralisation confirmation, quantitation of test organisms on carrier	AOAC Official Method 955.15 Testing Disinfectants against <i>Staphylococcus aureus</i> (ref 20).
Bactericidal	5% v/v serum option in additional guidance	Surface carrier (stainless steel)	<i>Pseudomonas aeruginosa</i> (ATCC 15442)	1×10^6 ; a 48 h-54 h liquid culture in broth is used	20°C, 10 min	Liquid culture of carriers: less than 1 positive out of 60 tested	<u>Controls:</u> Viability controls, verification of positive carriers, neutralisation confirmation, quantitation of test organisms on carrier	AOAC Official Method 964.02 Testing Disinfectants against <i>Pseudomonas aeruginosa</i> (ref 21).
Bactericidal veterinary use	Low-level soiling: 3 g/l bovine albumin High-level soiling: 10 g/l bovine albumin and 10 g/l yeast extract	Suspension	<i>Enterococcus hirae</i> (ATCC 10541), <i>Proteus vulgaris</i> (ATCC 13315), <i>Pseudomonas aeruginosa</i> (ATCC 15442), <i>Staphylococcus aureus</i> (ATCC 6538). Additional organisms possible	1.5×10^8 to 5×10^8 ; from a liquid culture in broth	10°C, 30 min Additional temperatures and times possible	At least 5 log reduction	<u>Controls:</u> 1. experimental conditions, 2. neutraliser or filtration, 3. method validation	EN 1656, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field — Test method and requirements (phase 2, step 1) (ref 12).
Fungicidal veterinary use	Low-level soiling: 3 g/l bovine albumin High-level soiling: 10 g/l bovine albumin and 10 g/l yeast extract	Suspension	<i>Candida albicans</i> (ATCC 10231), <i>Aspergillus niger</i> (ATCC 16404) (fungicidal). Additional organisms possible	1.5×10^7 to 5×10^7 ; suspension prepared from a culture on solid medium	10°C, 30 min Additional temperatures and times possible	At least 4 log reduction	<u>Controls:</u> 1. experimental conditions, 2. neutraliser or filtration, 3. method validation	EN 1657, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area — Test method and requirements (phase 2, step 1) (ref 15).

Sporicidal	Low-level soiling: 3 g/l bovine albumin	Suspension	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> (ATCC 6633). Additional organisms possible	1.5×10^6 to 5×10^6 ; suspension prepared from a culture on solid medium	20°C, 60 min Additional temperatures and times possible	At least 3 log reduction	No EN sporicidal test is available for veterinary use. If this protocol (EN 13704) is used, the soiling condition should be adjusted to high-level (10 g/l bovine albumin and 10 g/l yeast extract).	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) (ref 16).
Virucidal	Calf serum, other serum or pancreatic digest of casein (concentration not specified)	Surface (glass petri plates)	Vaccina WR strain (ATCC VR-119), Rotavirus Wa strain (ATCC VR-2018) Additional viruses possible	10^7 to 10^8 TCID ₅₀	22°C ± 2°C Additional temperatures possible Variable times	At least 4 log reduction, or 3 log reduction of viral titre beyond any disinfectant dilutions which exhibit cell culture cytotoxicity	<u>Controls</u> : cell culture control, virus control, cytotoxicity control, neutralisation control, other controls as required	ASTM E 1053-97 – Standard Test Method of Virucidal Agents Intended for Inanimate Environmental Surfaces (ref 13).
Virucidal	Low-level soiling: 3 g/l bovine albumin High-level soiling: 10 g/l bovine albumin and 10 g/l yeast extract	Suspension	Bovine Enterovirus Type 1 (ATCC VR-248)	$10^{7.5}$ TCID ₅₀	10°C, 30 min	At least 4 log reduction	EN 14675, Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area — Test method and requirements (phase 2, step 1) (ref 14).	

22 GUIDELINE FOR DATA TO SUPPORT EFFICACY OF DISINFECTANTS FOR VETERINARY USE

Bactericidal, Virucidal, Fungicidal, or Sporicidal	0.07% (w/v) Tryptone, 0.05% (w/v) BSA, 0.016% (w/v) Mucin	Surface carrier (stainless steel)	<i>Staphylococcus aureus</i> (ATCC 6538), <i>Pseudomonas</i> <i>aeruginosa</i> (ATCC 15442), <i>Trichophyton</i> <i>mentagrophytes</i> (ATCC 9533), <i>Bacillus subtilis</i> (ATCC 19659), Rotavirus Wa strain (ATCC VR- 2018), canine parvovirus Cornell strain (ATCC VR- 2017) Additional organisms possible	<i>S. aureus</i> 7 x 10 ⁶ , <i>P. aeruginosa</i> 7 x 10 ⁶ , <i>T. mentagro-</i> <i>phytes</i> 7 x 10 ⁴ , <i>B. subtilis</i> 7 x 10 ⁶	Variable	Variable performance standards apply	Elution from carrier, membrane filtration, incubation of filter on plate and colony count (NB: alternative protocol for viruses) <u>Controls:</u> carrier load, use of neutraliser optional. Additional controls required in virucidal tests.	ASTM E 2197-02, Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Myco- bactericidal and Sporicidal Activities of Liquid Chemical Germicides (ref 9).
---	---	--------------------------------------	---	---	----------	--	---	---

REFERENCES

1. APVMA Vet MORAG. Available at: http://www.apvma.gov.au/MORAG_vet/MORAG_vet_home.shtml.
2. APVMA Vet Labelling Code. Available at: http://www.apvma.gov.au/MORAG_vet/vol_5/vet_labelling_code.html.
3. Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP). Available at: <http://www.tga.gov.au/ndpsc/susdp.htm>.
4. First Aid Instruction and Safety Directions (FAISD) Handbook. Available at: <http://www.health.gov.au/internet/main/publishing.nsf/Content/ocs-faisd-handbook.htm>.
5. APVMA Operational Notice – Label claims for efficacy against pests and diseases which are exotic to Australia. Available at: http://www.apvma.gov.au/registration/morag/notices/docs/op_notice_exotics.pdf.
6. TGA Disinfectant Test. Available at: <http://www.tga.gov.au/docs/html/tgo/tgo54.htm>.
7. Antimicrobials Division US EPA – Initial virucidal effectiveness test using feline calicivirus as surrogate for norovirus. Available at: http://www.epa.gov/oppad001/pdf_files/initial_virucidal_test.pdf.
8. *Official Methods of Analysis of AOAC INTERNATIONAL* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **955.11**.
9. ASTM Standard E 2197 (2002) Standard quantitative disk carrier test method for determining the bactericidal, virucidal, fungicidal, mycobactericidal and sporicidal activities of liquid chemical germicides. ASTM International Designation E 2197-02.
10. ASTM Standard E 1054 (2008) Standard test methods for evaluation of inactivators of antimicrobial agents. ASTM International Designation E 1054-08.
11. Agricultural and Veterinary Chemicals Code Act 1994, Section 4, Definition of agricultural chemical product.
12. European Standard EN 1656 (2000) Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field — Test method and requirements (phase 2, step 1).
13. ASTM Standard E 1053-97 (1997) Standard test method of virucidal agents intended for inanimate environmental surfaces. ASTM International Designation E 1053-97.
14. European Standard EN 14675 (2006) Chemical disinfectants and antiseptics—Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in the veterinary area – test method and requirements (phase 2, step 1).
15. European Standard EN 1657 (2005) Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area — Test method and requirements (phase 2, step 1).
16. European Standard EN 13704 (2002) 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).
17. Kelsey JC and Maurer IM (1974) An improved (1974) Kelsey-Sykes test for disinfectants. *Pharmaceutical Journal (UK)* 213; 528–530.
18. Wright ES and Mundy RA (1960) Defined medium for phenol coefficient tests with *Salmonella typhosa* and *Staphylococcus aureus*. *Journal of Bacteriology* 80; 279–280.

19. MacKinnon IH (1974) The use of inactivators in the evaluation of disinfectants. *Journal of Hygiene (London)* 73; 189-195.
20. *Official Methods of Analysis of AOAC INTERNATIONAL* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **955.15**.
21. *Official Methods of Analysis of AOAC INTERNATIONAL* (2005) 18th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method **964.02**.