

Application for listing of a disinfectant as effective against bacterial and/or viral diseases of aquaculture relevance

PLEASE NOTE THIS IS A VOLUNTARY LISTING SCHEME AND NOT A STATUTORY AUTHORISATION

Please read these notes before completing this form.

- A. Applications will only be accepted from the manufacturer of the disinfectant. Listing is given for a product as manufactured by the applicant. A separate application form must be completed for each disinfectant.
- B. Applications will only be processed for products meeting the requirements of the Biocidal Products Directive; submission of this signed form signifies compliance with those requirements. Visit <http://ec.europa.eu/environment/biocides/index.htm> for details.
- C. Listing will be completed upon payment of the required fee by credit/debit card. Cash cannot be accepted. Fees for applications cannot be refunded regardless of the outcome.
- D. For listing purposes disinfectants are divided into two disease groups, bacterial and viral. Applications can be submitted for one or both of the disease groups, with a discounted fee for combined applications:
 - » Application fee for listing disinfectant as effective against viral diseases of aquaculture relevance £1355.00 excluding VAT.
 - » Application fee for listing disinfectant as effective against bacterial diseases of aquaculture relevance £1355.00 excluding VAT
 - » Combined application fee for listing disinfectant as effective against viral and bacterial diseases of aquaculture relevance £1855.00 excluding VAT
- E. Listing is granted for 5 years after which time it will be necessary to reapply for product listing.
- F. Please complete all relevant fields of this application form and the appropriate appendices to the listing required.
- G. In addition to the application form & appendices please enclose the following:
 - » Material safety data sheet (MSDS) for the product.
 - » Copy of the latest certificate of compliance for the testing laboratory to the required quality standard.
- H. For all successful applications, Cefas - on behalf of defra - will list on their web site www.efishbusiness.com:
 - » The manufacturers name and contact details.
 - » The product concerned & effective dilution rate of the product under the mandatory testing conditions.
 - » The effective dilution rate of the product under the optional testing conditions - where reported.

ALL INFORMATION IS TREATED AS COMMERCIAL IN CONFIDENCE WITH THE EXCEPTION OF THE INFORMATION SUPPLIED IN SECTION 5 WHICH WILL BE LISTED ON OUR WEBSITE FOR ALL SUCCESSFUL APPLICATIONS

CEFAS ACTING ON BEHALF OF DEFRA RESERVE THE RIGHT TO PERFORM CHECK-TESTS ON MARKETED PRODUCTS LISTED AS EFFECTIVE AGAINST BACTERIAL AND/OR VIRAL DISEASES OF AQUACULTURE RELEVANCE. PRODUCTS FOUND TO BE INEFFECTIVE AT A DILUTION LISTED AS EFFECTIVE AGAINST VIRAL OR BACTERIAL PATHOGENS WILL BE REMOVED FROM LISTING

1. Information required for all applications

1.1 Name of manufacturing company's representative¹

1.2 Name and address of manufacturing company

1.3 Contact telephone number¹
(incl. International dialling code where appropriate)

1.4 Contact email address¹

1.5 Contact fax number¹

1.6 Company VAT number

1.7 Application payment reference

There may be other representatives we can communicate with and the names of these should be made know to us at your earliest convenience.

¹ To safe-guard information about your product this is the person Cefas will use in all correspondence. This should be the person who signs this form.

2. Parent production information

2.1 Enter your unique parent product name

2.2 Physical form in which the disinfectant was tested and is to be sold

2.3 Total product composition including; dyes, perfumes, etc *(Use additional sheet(s) if necessary)*

Compound Name	CAS Number	% active in raw material	% raw material in final product	Active biocide or inert substance

Please tick if additional sheet(s) were used for the information provided in 2.4

3. Complete this table for new products listings/renewals only, i.e. not additional trade name applications for products with a valid listing

Please complete this table for 'parent' products indicating which application you are applying for listing under, the dilution and the fee being paid.

(1) Test for listing use	(2) Tick which types of listing are sought	(3) Dilution at which the preparation is to be listed as effective (liquids expressed as 1 part to X parts water; solids expressed as 1g to X mls of water)	(4) Fee for Individual applications (£)	(5) Fee for Combined applications (£)
EN 1656:2000 modified - Effectiveness against bacterial diseases of aquaculture relevance				
EN 14675:2006 modified - Effectiveness against virological diseases of aquaculture relevance				

4. Complete this table for trade name (back to back) listings only

- 4.1 Please complete the following table indicating the listing under which the parent product is pending or already listed for use under, the listed dilution and the date (if applicable) that the product was listed.

Aquaculture Listing	Effective Dilution as Listed (liquids expressed as 1 part to X parts water; solids expressed as 1g to X mls of water)	Date of Listing
EN 1656:2000 modified - effectiveness against bacterial diseases of aquaculture relevance		
EN 14675:2006 modified - effectiveness against viral diseases of aquaculture relevance		

5. Company and product details for website listing

- 5.1 Manufacturers of a listed disinfectant may make it available under different trade names or supply it to customers wishing to make it available under their own trade names. The disinfectant listed and supplied must be identical to the sample tested. Please give the trade names already used for your product as well as new trade names for which listing is being sought. Also provide the names and addresses of companies wishing to distribute your product under their own trade names. A disinfectant must not be marked as listed until its name appears on the list published by Cefas on behalf of defra at www.efishbusiness.co.uk. *(Use additional sheet(s) if necessary)*

Trade names to appear on the list published on the web site	Company name	Address, email and telephone number and company website	Name and signature of company representative

Please tick if any information has been supplied separately

Listing of disinfectants by Cefas on behalf of defra is a voluntary scheme, which does not relieve manufacturers of their obligation to obtain other statutory or non-statutory clearance, or of their general responsibilities to users of their products. Attention is also drawn to legal requirements regarding classification, packaging, labelling and provision of information on dangerous goods, both for supply and transport. Advice on these may be sought from the Health and Safety Executive www.hse.gov.uk

Cefas acting on behalf of defra reserve the right to perform check-tests on marketed products listed as effective against bacterial and/or viral diseases of aquaculture relevance. Products found to be ineffective at a dilution listed as effective against viral or bacterial pathogens will be removed from listing

Company representative's signature (person named at 1.1)

Signed on behalf of (Company name)

Date:

Confidentiality of data

Commercial information supplied to defra, Cefas and technical experts involved in the processing of listing applications will be treated as confidential. Any personal data supplied on this form or provided separately by you in connection with this application will be used to contact you and process your application. Such data will be stored on a database for the purpose of administering the disinfectant listings mechanism. Names and addresses of manufacturers and distributors of disinfectants listed for use as effective against bacterial and/or viral diseases of aquaculture relevance will be publicly available on the efish business website at www.efishbusiness.co.uk and in hard copy form in a list obtainable from the Cefas Disinfectant Listing Team, contact address detailed below.

Send the application form, appendices (or equivalent) & test reports by post to:

Disinfectant Listing Team
Cefas – Weymouth Laboratory
Barrack Road, The Nothe,
Weymouth, Dorset
DT4 8UB

Receipt of your application form will usually be acknowledged within 5 working days.

Please complete the checklist below to confirm that the application form and appropriate appendices (or equivalent) have been fully completed and that the additional mandatory information has been enclosed in support of this application:

- | | |
|--|--------------------------|
| Application form completed | <input type="checkbox"/> |
| Appendix 2 - Example Modified EN1656 Quality Assurance Checklist (as attached or equivalent if using own quality assurance checklist) | <input type="checkbox"/> |
| And/or | |
| Appendix 4 - Example Modified EN14675 Quality Assurance Checklist (as attached or equivalent if using own quality assurance checklist) | <input type="checkbox"/> |
| MSDS Enclosed | <input type="checkbox"/> |
| EN 1656 Test report (see appendix 5) | <input type="checkbox"/> |
| And/or | |
| EN 14675 Test report (see appendix 6) | <input type="checkbox"/> |
| Copy of latest certificate of compliance for the testing laboratory to recognised quality standard | <input type="checkbox"/> |

Please note that failure to submit all information as requested will result in the return of your application without further processing and additional fees may be charged in order to resubmit an application.

Appendix 1 - Guideline for listing a product as effective against bacterial diseases of aquaculture relevance

In support of listing a product dilution as being effective against bacterial diseases of aquaculture relevance, the product must be tested using a modification of EN standard 1656, (Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field) as described below. The results, demonstrating that the reported effective dilution passed the aforementioned test must be submitted to Cefas with this application (reference Appendix 5).

Modifications of EN1656: 2000 to be followed

1. The test organisms recommended in the standard (Section 5.2.1. and all references thereafter) must be substituted for: *Aeromonas salmonicida* sub sp. *salmonicida* (ATCC 14174), *Yersinia ruckeri* (ATCC 29473), *Carnobacterium maltaromaticum* (syn *C. piscicola*) (ATCC 35586) and *Lactococcus garvieae* (NCIMB 702927).
2. The recommended temperature at which test plates are incubated prior to enumeration of viable colonies shall be changed from 36 or 37°C to 22°C ± 1°C (Section 5.3.2.3 and all references thereafter).
3. Choice of experimental conditions (Section 5.5.1) mandatory conditions:
 - a. For all dilutions, the temperature to be tested is 4 °C ± 1 °C (not 10 °C).
 - b. For all dilutions, the contact time to be tested is 30 min ± 10 sec *.
 - c. For all dilutions, the Interfering substance to be tested is as described for dirty conditions (10g/L yeast extract plus 10g/L bovine serum albumin solution).
4. Optional data (Section 5.5.1):
 - a. The product performance at 20 °C can also be reported to Cefas.
 - b. The product performance at a contact time of 5 min ± 10 sec * can also be reported to Cefas at both 4°C and 20 °C.
 - c. For all dilutions, the Interfering substance to be tested is as described for dirty conditions (10g/L yeast extract plus 10g/L bovine serum albumin solution).

* Although the standard specifies contact times should be ± 10 sec, and testing laboratories should endeavour to adhere to this requirement, reported deviations of ± 30 sec will not be regarded as an invalidation of the reported test results. Deviations ± > 30 seconds will invalidate a reported test result.

If any problems arise following this standard the Cefas Listing Administration Team should be contacted for advice.

Quality Assurance

1. Testing laboratory. The laboratory performing the testing must adhere to a recognised International laboratory quality system (e.g. ISO 17025, Good Laboratory Practice or Good Manufacturing Practice), please contact Cefas Listing Administration Team for further guidance.
2. Audited data. In support of the product dilution that is claimed to be effective against aquaculture pathogens, test results must be accompanied by an audit report affirming that the test was done to the conditions specified in the standard. To assist this process, a Quality Assurance (QA) checklist is supplied with this guideline (Appendix 2). The QA auditor completing and signing off the QA checklist must be independent to the test department and with experience of quality assurance, such as a Quality or Technical Manager.

Reporting

Test results are to be submitted with this application to Cefas in the format suggested by EN 1656: 2000 Section 5.8 (see Appendix 5 of this form), with the dilution that the company concerned wishes to report as being an effective concentration, and one other concentration, shown to pass the test under the recommended conditions in the test report (demonstrate a 10⁵ or more reduction in viability within 30 min at 4 °C in the presence of 10g/L yeast extract plus 10g/L bovine serum albumin solution) for all four of the test organisms (Section 1 above). Test results for at least one concentration that failed the test should also be included. For reporting optional data (Section 4 above), this should be done in a similar format as that for reporting dilutions effective under the mandatory testing conditions. It should be noted that failure to report results using the format suggested (EN 1656:2000 Section 5.8), including the results of required validation exercises, may result in the application not being accepted by Cefas.

Listing

Cefas on behalf of defra will list on their website:

- The product concerned
- The dilution that the company wishes to have listed as being effective against aquaculture relevant bacterial pathogens under the mandatory testing conditions
- Dilutions of the product demonstrated to be effective against aquaculture relevant bacterial pathogens under the optional testing conditions
- Links to product relevant information supplied by the manufacturers (note, this will include a disclaimer emphasizing that supplying a link through the Cefas website does not mean that Cefas/defra approves or condones any claims made by the site referred to). Cefas and defra do not accept liability for any products listed under this scheme on their website.

References

British Standards Institution (BSi), 2000. BS 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). BSi British Standards London UK pp. 34, <http://www.bsi-global.com/en/>

British Standards Institution (BSi), 2006. BS EN 12353:2006 Chemical disinfectants and antiseptics. Preservation of test organisms used for the determination of bactericidal, sporicidal and fungicidal activity. BSi British Standards, London UK pp. 26, <http://www.bsi-global.com/en/>

Verner - Jeffreys DW, Ridout N, Joiner C, Reese RA, Husby A & Dixon PF (2009) Development of virucidal and bactericidal aquaculture disinfectant testing standards. *Aquaculture* 286, 190-197.

Appendix 2 - Example Modified EN 1656:2000 Quality Assurance Checklist

Quality Assurance Checklist for Modification of EN1656 (Evaluation of Bactericidal activity of chemical disinfectants and antiseptics used in veterinary field)				
Reference	Requirement	Satisfactory		Comment
		Yes	No	
Test substance				
Name	Listed in application			
Nominal Active Ingredient concentration	Listed in test report			
Batch Number	Listed in test report			
Manufacturer	Listed in test report			
Expiry Date	Listed in test report			
Storage	Listed in test report			
EN1656 5.4.2	Product test solutions prepared freshly in standardized hard water and used within 120 min.			
Test organisms				
EN1656 5.2.1 modification 1	<i>A. salmonicida</i> ATCC 14174 <i>Y. ruckeri</i> ATCC 29473 <i>Carnobacterium maltaromaticum</i> (syn <i>C. piscicola</i>) ATCC 35586 <i>Lactococcus garviae</i> (NCIMB 702927) (Stored frozen at less than -70°C using BS EN 12353:2006 recommended methods or cryopreservative beads)			
EN1656 5.4.1.2	24 hour second or third subcultures from the stock culture must be used. A third subculture may be prepared from a 48-hour second subculture if necessary.			
EN1656 5.4.1.3	Cell suspensions prepared to between 1.5×10^8 and 5×10^8 cfu/ml			
EN1656 5.4.1.3	Prepared suspensions maintained in water bath at $20^\circ\text{C} \pm 1^\circ\text{C}$ and used within 2 hours			
EN1656 5.4.1.3 modification 2	Dilutions prepared to 10^{-6} and 10^{-7} for counting and pour plates incubated at $22^\circ\text{C} \pm 1^\circ\text{C}$			
EN1656 A 4.1.2 c)	Prepare validation suspension (Nv) of 6×10^2 to 3×10^3 cfu/ml (approximately 10^{-5})			

Quality Assurance Checklist for Modification of EN1656 (Evaluation of Bactericidal activity of chemical disinfectants and antiseptics used in veterinary field)

Reference	Requirement	Satisfactory		Comment
		Yes	No	
EN1656 A.2	Prepare bacterial suspension, 10 ⁻¹ dilution with the diluent in duplicate and inoculate using pour plate method (10 ⁻² dilution may also be prepared)			
Culture media and reagents				
EN1656 5.2.2.1	Reagents appropriate for microbiological purposes			
EN1656 5.2.2.2	Water, either glass distilled or adhering to criteria for injectable preparations (European Pharmacopoeia)			
EN1656 5.2.2.3	Media used for maintenance and viable counts is Tryptone soy agar			
EN1656 5.2.2.4	Diluent used is Tryptone Sodium Chloride solution.			
EN1656 5.2.2.5	Neutralizer validated in accordance with annex A			
EN1656 5.2.2.7	Standardized Hard water freshly prepared on the day			
Experimental conditions				
EN 1656 5.5.1a modification 3a	Contact temperature to be tested is 4°C ± 1°C			
EN 1656 5.5.1a modification 4a	Optional additional performance data at 20°C may be reported			
EN 1656 5.5.1b modification 3b	Contact time to be tested is 30 min ± 10 sec			
EN 1656 5.5.1b modification 4b	Optional data for 5 minute contact time may be reported			
EN1656 5.5.1d modification 3c	Interfering substance as for high level soiling (10g/L yeast extract+10g/L bovine albumin) used for all dilutions			
Dilution-neutralization method				
EN1656 5.5.2.2.1	Equilibrate water and neutraliser to 20°C ± 1°C and all other reagents to test temperature			
EN1656 5.5.2.2.2	Pipette 1 ml interfering substance and 1 ml bacterial suspension into test tube			
EN1656: 2000 5.5.2.2.2	Avoid touching upper part of test tube sides when adding bacteria			
EN1656 5.5.2.2.2 and modification 3a	Immediately start stopwatch, mix and place in water bath at 4°C ± 1°C			
EN1656 5.5.2.2.2	After 2 min ± 10 sec add 8 ml of product test solution			

Quality Assurance Checklist for Modification of EN1656 (Evaluation of Bactericidal activity of chemical disinfectants and antiseptics used in veterinary field)

Reference	Requirement	Satisfactory		Comment
		Yes	No	
EN1656 5.5.2.2.2	Restart stopwatch, mix and return to water bath for 30 mins ± 30 sec			
EN1656 5.5.2.2.2	Mix just before end of contact time			
EN1656 5.5.2.2.2	At end of contact time pipette 1 ml mixture into tube containing 8 ml neutralizer and 1 ml water in a water bath at 20°C ± 1°C			
EN1656 5.5.2.2.2	After 5 min ±10 sec transfer 2 x 1 ml neutralized mixture into Petri dishes			
EN1656 5.5.2.2.2	Quickly add 12 – 15 ml melted TSA cooled to 45°C ± 1°C (in exceptional cases neutralizer can be added to TSA)			
EN1656 5.5.2.2.3 modification 2	Incubate at 22°C ± 1°C for 24 hours			
EN1656 5.5.2.2.3	Determine cfu for each plate and incubate for a further 24 hours			
EN1656 5.5.2.2.3	Recount only plates showing well separated colonies			
EN1656 5.5.2.2.3	Using highest number of colonies for each plate calculate cfu/ml in test mixture according to EN1656 5.6.1.2			
Dilution-neutralisation method validation				
Validation of dilution-neutralization method	Done for each test strain according to EN1656:2000 annex A			
EN1656 annex A 4.1.2 c) (modification 3a)	1 ml interfering substance, 1 ml diluent and 8 ml strongest product dilution placed in sterile tube and transferred to water bath at 4°C			
EN1656 annex A c)	1 ml of the mixture placed into tube containing 8 ml neutralizer in water bath at 20°C and left for 5 min ± 10 sec			
EN1656 annex A c)	Add 1 ml of prepared validation suspension (<i>M_v</i>) and immediately start stopwatch			
EN1656 annex A c)	Vortex and return to 20°C water bath for 30min ± 1 min.			
EN1656 annex A c)	Vortex immediately before end of contact time			
EN1656 annex A c)	Take 1 ml of sample in duplicate and prepare plates in duplicate using pour plate method.			

Quality Assurance Checklist for Modification of EN1656 (Evaluation of Bactericidal activity of chemical disinfectants and antiseptics used in veterinary field)

Reference	Requirement	Satisfactory		Comment
		Yes	No	
EN1656 annex A modification 2	Incubate at 22 ± 1°C for 24 hours			
EN1656 annex A modification 2	Count then incubate at 22 ± 1°C for a further 24 hours and re-count.			
EN1656 annex A c)	Use highest number of cfu/plate to calculate viable count using method given in EN1656 5.6.1.2			
Calculation and expression of results				
EN1656 5.6.1.1	Only colony counts of less than 300 to be used for calculation of viable count			
EN1656 5.6.1.1	At least one of each plate set must have 15 or more colonies			
EN1656 5.6.2	<p>Verification of methodology For each test organism check that:</p> <p>a) N is between 1.5×10^8 cfu/ml and 5×10^8 cfu/ml b) Nv is between 6×10^2 cfu/ml and 3×10^3 cfu/ml c) A is equal to or greater than 0.05 times Nv d) B is equal to or greater than 0.05 times Nv e) C is equal to or greater than 0.5 times B</p> <p>where: N is the number of cfu/ml of the bacterial test suspension (see 5.4.1.4) Nv is the number of cfu/ml of the bacterial suspension (see A.2) A is the number of cfu/ml of the experimental conditions validation (see A.4.1.2a) or (see A.4.2.2a) B is the number of cfu/ml of the neutralizer toxicity validation (see A.4.1.2 b) or of the filtration control (see A.4.2.2 b) C is the number of cfu/ml of the dilution-neutralization validation (see A.4.1.2 c) or of the filtration test control (see A.4.2.2 c)</p>			
EN1656 5.6.3	For each organism and product test concentration reduction in viability calculated using formula given in EN1656 5.6.3			
Conclusion and test report	The product is deemed to have passed the test if it demonstrates a 10^5 or more reduction in viability within 30 min or less using the specified organisms and test conditions			

Quality Assurance Checklist for Modification of EN1656 (Evaluation of Bactericidal activity of chemical disinfectants and antiseptics used in veterinary field)				
Reference	Requirement	Satisfactory		Comment
		Yes	No	
EN1656 5.8	The test report shall refer to the EN1656 standard and shall include the information specified at EN1656 5.8 (see appendix 5 of this form)			

Complete all boxes as appropriate and add full comments for any non-satisfactory marking.

Note - if the membrane filtration method, rather than neutralization dilution, is employed the checklist must be modified as appropriate to show the additional required steps and test procedures and all references to neutralization dilution marked N/A (not appropriate).

Any operation not included in the standard as well as any incident that may affect the results should be reported in full.

Test conducted on: (Date)

By: (Tester signature)

Print name:

Quality control countersignature:
(e.g. test department Technical Manager)

Date:

Print name:

Quality Assurance Statement

This test was/was not (delete as applicable) conducted in accordance with the conditions specified in the Standard and guidelines described in this Appendix.

Quality Assurance Auditor signature:

Date:

Print name:

Appendix 3 - Listing a product as effective against viral diseases of aquaculture relevance

In support of listing a product dilution as being effective against viral diseases of aquaculture relevance, the product must be tested using a modification of EN standard 14675:2006, (Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in veterinary field) as described below. The results, demonstrating that the reported effective dilution passed the aforementioned test must be submitted to Cefas with this application (reference Appendix 6).

Modifications of EN14675: 2006 to be followed

1. The test organisms recommended in the standard (Section 4/5.2.1 and all references thereafter) should be substituted for: Infectious pancreatic necrosis virus (IPNV), serogroup A2, Spjarup (SP) isolate (ATCC VR-1318).
2. Choice of experimental conditions (Section 5.5.1.1) mandatory conditions:
 - a. For all dilutions, the temperature to be tested is $4\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ (not $10\text{ }^{\circ}\text{C}$).
 - b. For all dilutions, the contact time to be tested is $30\text{ min} \pm 10\text{ sec}^*$.
 - c. For all dilutions, the Interfering substance to be tested is as described for dirty conditions (10g/L yeast extract plus 10g/L bovine serum albumin solution).
3. Optional data (Section 5.5.1):
 - a. The product performance at $20\text{ }^{\circ}\text{C}$ can also be reported to Cefas.
 - b. The product performance at a contact time of $5\text{ min} \pm 10\text{ sec}^*$ can also be reported to Cefas.

* Although the standard specifies contact times should be $\pm 10\text{ sec}$, and testing laboratories should endeavour to adhere to this requirement, reported deviations of $\pm 30\text{ sec}$ for all times in the standard will not be regarded as an invalidation of the reported test results. Deviations $\pm > 30\text{ seconds}$ will invalidate a reported test result.

As stated in the standard, additional or alternative specific virucidal activity can be determined applying other contact times, temperatures and test organisms in accordance with 5.2 and 5.5.1.1 in order to take into account intended specific use conditions, although in this case validation data should accompany the test result, justifying the employment of the alternative conditions, for review by Cefas.

Section 5.5.1.2. As routine production of $> 10^{7.5}$ TCID₅₀ IPNV can be problematical, lower concentrations can be used in the assay providing a 4- log₁₀ reduction can be derived from the results.

4. The preparation of hard water should be amended if ANALAR grade chemicals are used.
5. MDBK cells, used to multiply the virus (Section 5.5.1.2), should be replaced by Chinook salmon embryo (CHSE-214, ATCC CRL 1681) cells or alternative cell lines dependent on the virus to be tested.
6. Cells may be attached to the plate as a confluent monolayer of cells rather than in suspension (Section 5.5.1.3). If this is the case cell culture techniques may differ.
7. Virus titrations/dilutions may be made on the microtitre plate if plates contain a monolayer of cells (rather than in separate containers then transferred to the plate as in section 5.6). When this is the case the neutralising dilutions will not be made in ice cold diluent (Section 5.1) and the time between removing the test mix from the water bath and neutralising dilution should be $\pm 30\text{s}$.
8. Titrations may be made with ≥ 6 wells per dilution (Standard states 8 wells per dilution section 5.6.2 and 5.8).
9. Dilution is used to stop the action of the test product therefore a control test is needed to confirm this works.
10. Dilute the product 1/10 and 1/100 then add the virus for 30 min at $4\text{ }^{\circ}\text{C}$ before titration. The titre of the virus should not decrease, as the product should have been inactivated by the dilution before the virus is added. If the product fails this test alternative neutralisers will have to be tested.
11. Titration of test virus suspension (Annex A.3) states '1 ml of hard water is to be used instead of the product test solution'. 8 ml of product test solution is used throughout the tests therefore it should be 8 ml used in this test.
12. The test virus reference inactivation test using formic acid (Annex A.2) is not necessary. Delete also 3.9 (under terms and definitions) and 7.1 d) Test results: virus inactivation of the reference virus inactivation test after 30 min.
13. Treatment of cells (Annex A.1) in the standard the control uses PBS but maintenance medium should be used.
14. The level of cytopathic effect (CPE) recorded (section 6.1) is not necessary, only record + if CPE is present or – if no

CPE.

15. Test virus suspension shall not be less than $10^{7.0}$ TCID₅₀ per ml
16. 5.3.2.3 substitute 15 °C ± 1°C for 37 °C (temperature for incubating CHSE-214 cells and all other references to incubating CHSE-214 cells).
17. 5.3.2.11 Suggest 6-well multiplates are used here instead of petri dishes
18. 5.6.3 Plaque assay. Substitute 20 °C ± 1°C for virus absorption temperature and 15 °C for all subsequent incubation temperature. Low gelling temperature agarose should also be used. Neutral Buffered Formalin can be used for fixation.

If any problems arise following this standard (e.g. cell cytotoxicity). The Cefas Listing Administration Team should be contacted for advice.

Quality Assurance

1. Testing Laboratory. The laboratory performing the testing must adhere to a recognised International laboratory quality system (ISO 17025, Good Laboratory Practice or Good Manufacturing Practice), please contact Cefas Listing Administration Team for further guidance.
2. Audited data. In support of the product dilution that is claimed to be effective against aquaculture pathogens, test results must be accompanied by an audit report affirming that the test was done to the conditions specified in the standard. To assist this process, a Quality Assurance (QA) checklist is supplied with this guideline (Appendix 4). The QA auditor completing and signing off the QA checklist must be independent to the test department and with experience of quality assurance, such as a Quality or Technical Manager.

Reporting

Test results are to be submitted with this application to Cefas in the format suggested by EN 14675:2006 Section 7.1 and Annex C. The results should include details of all relevant test results, including testing for cytotoxicity caused by product solutions (Annex A.1) and results of titration of test virus suspension (Annex A.3) (See Appendix 6 in this form), with the dilution that the company concerned wishes to report as being an effective concentration, and one other concentration, shown to pass the test under the recommended conditions in the test report (demonstrate a 10^4 or more reduction in viability within 30 min at 4 °C in the presence of 10g/L yeast extract plus 10g/L bovine serum albumin solution). Test results for at least one concentration that failed the test are also to be included. For reporting optional data (Section 3), this should be done in a similar format as that for reporting dilutions effective under the mandatory testing conditions. It should be noted that failure to report results using the format suggested (EN 14675: 2006 Section 5.8), including the results of required validation exercises, may result in the application not being accepted by Cefas.

Listing

Cefas on behalf of defra will list on their website:

- The product concerned.
- The dilution that the company wishes to have listed as being effective against aquaculture relevant viral pathogens under the mandatory testing conditions.
- Dilutions of the product demonstrated to be effective against aquaculture relevant viral pathogens under the optional testing conditions.
- Links to product relevant information supplied by the manufacturers (note, this will include a disclaimer emphasizing that supplying a link through the Cefas website does not mean that Cefas/defra approves or condones any claims made by the site referred to). Cefas and defra do not accept liability for any products listed under this scheme on their website.

References

British Standards Institution, 2006. BS EN 14675:2006 Chemical disinfectants and antiseptics- Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in veterinary field- test method and requirements (Phase 2 step 1). BSi British Standards, London UK pp. 28, <http://www.bsi-global.com/en/>

Verner - Jeffreys DW, Ridout N, Joiner C, Reese RA, Husby A, Dixon PF. (2009) Development of virucidal and bactericidal aquaculture disinfectant testing standards. *Aquaculture* 286, 190-197.

Appendix 4 - Example Modified EN14675:2006 Quality Assurance Checklist

Quality Assurance Checklist for Modification of EN14675 (Evaluation of Virucidal activity of chemical disinfectants and antiseptics used in veterinary field)					
	Reference	Requirement	Satisfactory		Comment
			Yes	No	
Test substance:					
Name		Listed in application			
Nominal Active Ingredient concentration		Listed in test report			
Batch Number		Listed in test report			
Manufacturer		Listed in test report			
Expiry Date		Listed in test report			
Storage		Listed in test report			
Concentration	EN 14675:2006	Shall be 1.25 times the desired test concentration			
Test solutions	EN 14675:2006	Prepared freshly in hard water (water for ready to use product)			
Test solutions	EN 14675:2006	Used in the test within 2 hours			
Experimental conditions:					
Temperature		4°C ± 1°C			
Contact time		30 min ± 30sec			
Interfering substance (High level soiling only)		10g/L bovine albumin plus 10g/L yeast extract			
Test agent		IPNV A2 Sparjup			
Additional test agent		Listed in test report			
Water (for culture media)		Produced by reverse osmosis			
Hard water (for dilution of product)		Prepared using ANALAR reagents			
Cell culture medium		Any medium demonstrably suitable for culture of CHSE-214 cells (eg L-15+10%FBS)			
Preparation of Microtitre plates		Cell suspensions prepared by trypsinising with 0.25% trypsin (EDTA) solution			

Quality Assurance Checklist for Modification of EN14675 (Evaluation of Virucidal activity of chemical disinfectants and antiseptics used in veterinary field)					
	Reference	Requirement	Satisfactory		Comment
			Yes	No	
Preparation of Microtitre plates		Cells split at 1:3 ratio in cell culture medium +10 % foetal calf serum (FCS) to enable formation of monolayer in two days			
Viral suspension dilutions/titration		Done in the plate, 6 wells per dilution			
Pre-test for cytotoxicity					
EN 14675:2006	A.1	Solutions prepared as for the test using hard water instead of virus and inoculated into monolayer cell cultures			
EN 14675:2006	A.1	Comparative virus titrations performed on cells that have not been treated with disinfectants			
EN 14675:2006	A.1	Only dilutions showing a low degree of cell destruction or produce virus titre reduction $\log_{10} < 1.0$ used for determination of residual infectivity			
		Record +ve results as '+' and wells without CPE as '-'			
Test method					
EN 14675:2006	10.0	Vortex just before end of contact time			
EN 14675:2006	5.1	0.5 ml virus/disinfectant taken after 30 min (+/- 30 sec) contact time			
EN 14675:2006	10.0	Add 0.5ml of test mixture to 4.5ml minimum essential medium (MEM) + 5% FCS at $4 \pm 1^\circ\text{C}$			
EN 14675:2006	10.0	Prepare dilutions to 10^{-8} in MEM + 2% FCS and keep at $4 \pm 1^\circ\text{C}$			
EN 14675:2006	10.0	≥ 6 wells of confluent monolayer of cells in micro titre plates inoculated with each dilution			
EN 14675:2006	10.0	Titre of infectivity calculated after incubation by formula described by Karber 1931 ¹			
Verification					
EN 14675:2006	6.4 a)	Test is valid if $4 \log_{10}$ reduction after treatment			
EN 14675:2006	6.4 c)	The cytotoxicity of the product solution does not affect cell morphology or virus susceptibility so as to compromise demonstration of $4 \log_{10}$ reduction in virus titre			

¹ Kärber, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Archiv für Experimentelle Pathologie und Pharmakologie, 162: 480-483.

Quality Assurance Checklist for Modification of EN14675 (Evaluation of Virucidal activity of chemical disinfectants and antiseptics used in veterinary field)

	Reference	Requirement	Satisfactory		Comment
			Yes	No	
EN 14675:2006	6.4 d)	Comparative virus titration on cells inoculated with test mixture or virus dilutions result in a difference of $\log_{10} < 1$ of virus titre			
EN 14675:2006	7.1	Product deemed to have passed if demonstrates $\geq 4 \log_{10}$ reduction in titre within 30 min at 4°C under the defined test conditions			

Reporting of results

EN 14675:2006	6.6	Results tabulated as raw data and also expressed as negative logarithmic values of 50% tissue culture infectious dose (TCID ₅₀)			
EN 14675:2006	6.6	If no virus multiplication in highest concentration titre result calculated as < lowest dilution used, and if there is virus multiplication in all dilutions, result calculated as > highest dilution used			

The report shall include:

		<p>The test report should refer to the EN14675:2006 standard and shall include the information specified in EN14675:2006 Section 7.2 (see appendix 6 of this form) and shall also include:-</p> <ul style="list-style-type: none"> • Diluent to stop action of product 			
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Complete all boxes as appropriate and add full comments for any non-satisfactory marking.

Any operation not included in the standard, as well as any incident that may affect the results, should be reported in full.

Test conducted on: (Date)

By: (Tester signature)

Print name:

Quality control countersignature:
(e.g. test department Technical Manager)

Date:

Print name:

Quality Assurance Statement

This test was/was not (delete as applicable) conducted in accordance with the conditions specified in the Standard and guidelines described in this Appendix.

Quality Assurance Auditor signature:

Date:

Print name:

Appendix 5 - Model Test Report Format for Standard EN 1656 (reference European Standard EN 1656:2000 Section 5.8)

Determination of veterinary bactericidal activity in high soiling conditions.

- a. Identification of the test Laboratory:
- b. Identification of the sample:
 - i. Name of the product:
 - ii. Batch number:
 - iii. Manufacturer:
 - iv. Date of delivery:
 - v. Expiry date:
 - vi. Storage conditions:
 - vii. Product diluent recommended by the manufacturer for use:
 - viii. Active substance(s) and its/their concentrations (optional):
 - ix. Appearance of the product:
- c. Test method and its validation:
 - i. If the dilution-neutralization method is used full details of the tests for validation of the neutralizer shall be given:
 - ii. If the membrane filtration method is used full details of the tests for validation of the method shall be given. Full details of the procedure which was carried out to justify the use of the membrane filtration method shall also be given:
- d. Experimental conditions:
 - i. Period of analysis:
 - ii. Appearance of the product dilutions:
 - iii. Product test concentrations:
 - iv. Test temperature(s):
 - v. Contact time(s):
 - vi. Interfering substance:
 - vii. Product diluent:
 - viii. Stability of test mixture:
 - ix. Temperature of incubation:
 - x. Counting procedure:
 - xi. Neutralizer or rinsing liquid
 - xii. Identification of bacterial strains used:
- e. Test results, to include validation tests and evaluation of bactericidal activity:
 - i. Please attach test result tables, numbering each page.
 - ii. Please state no. of results pages attached:
- f. Special remarks:
- g. Conclusion:

Completed at:
(Address of testing laboratory)

Signature:
Date:

Print Name:

Appendix 6 – Model Test Report Format for Standard EN14675 (reference European Standard EN 14675:2006 Section 7.2)

Determination of veterinary virucidal activity in high soiling conditions.

- a. Identification of the test laboratory:
- b. Identification of the sample:
 - i. Name of the product:
 - ii. Batch number:
 - iii. Manufacturer:
 - iv. Date of delivery:
 - v. Expiry date:
 - vi. Storage conditions:
 - vii. Product diluent recommended by the manufacturer for use:
 - viii. Active substance(s) and its/their concentrations (optional):
 - ix. Appearance of product:
- c. Experimental conditions:
 - i. Period of analysis (dates of test):
 - ii. Appearance of product dilutions:
 - iii. Diluent used for product test solution (hard water or distilled water):
 - iv. Product test concentrations:
 - v. Test temperature(s):
 - vi. Contact time(s):
 - vii. Interfering substances:
 - viii. Stability and appearance of the mixture during the procedure (note the formation of any precipitate or flocculent):
 - ix. Temperature of incubation:
 - x. Identification of viral strain used:
- d. Test results, to include validation of test results, titre of virus suspension, maximum detectable virus inactivation, virus inactivation of the reference virus inactivation test after 30 min:
 - i. Please attach test results, numbering each page.
 - ii. Please state no. of results pages attached:
- e. Special remarks:
- f. Conclusion:

Completed at:
(Address of testing laboratory)

Signature:

Print Name:

Date: