Department for Environment Food & Rural Affairs

Centre for Environment Fisheries & Aquaculture Science

Application for listing 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

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 1656:2019 (Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in the veterinary area) 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 EN1656:2019 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, NCIMB 833), *Yersinia ruckeri* (ATCC 29473, NCIMB 2194), *Carnobacterium maltaromaticum (*syn *C. piscicola)* (ATCC 35586, NCIMB 2264) 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. Experimental conditions (Section 5.5.1) mandatory conditions:
    a. For all dilutions, the temperature to be tested is 4 °C ± 1 °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 high level soiling conditions (10g/L yeast extract plus 10g/L bovine serum albumin solution).
4. Optional data
    a. The product performance at 20 °C ± 1 °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 4ºC and 20 ºC.

* 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 for all times in the standard will not be regarded as an invalidation of the reported test results. Deviations ± > 30 seconds will invalidate a reported test result.

5. Incubation and counting of the test mixture and the control and validation mixtures (Section 5.5.2.6a). Due to the slower bacterial growth colonies can be incubated for a continuous 40-48 hours without the mid cfu count after 20-24 hours.
6. Prepared validation suspension (*Nv*) is acceptable between (3.0×10^2) cfu/ml and (3.0×10^3) cfu/ml (Section 5.4.1.5 and 5.7.3). If any problems arise following this standard the Cefas Disinfectant 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 Disinfectant Listing 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 of 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 1656:2019 Section 5.10 (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 10^5 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 (Modification 1 above). Test results for at least one concentration that failed the test should also be included. For reporting optional data (Modification 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 1656:2019 Section 5.10), 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), 2019. BS EN 1656:2019 Chemical disinfectants and antiseptics- Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area - Test method and requirements (Phase 2 step 1). BSi British Standards London UK, http://www.bsi-global.com

British Standards Institution (BSi), 2006. BS EN 12353:2021 Chemical disinfectants and antiseptics. Preservation of test organisms used for the determination of bactericidal, sporicidal and fungicidal activity. BSi British Standards, London UK, http://www.bsi-global.com/en/.

Verner-Jeffreys DW, Ridout N, Joiner C, Reese RA, Husby A & Dixon PF (2019) Development of virucidal and bactericidal aquaculture disinfectant testing standards. Aquaculture 286, 190-197.

Appendix 2 - Example Modified EN 1656:2019 Quality Assurance Checklist

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			
Legal status	EU Biocides Regulation compliant			
Expiry date	Listed in test report			
Storage	Listed in test report			
Test organisms:				
EN1656:2019 5.2.1 and modification 1	*A. salmonicida* ATCC 14174, NCIMB 833 *Y. ruckeri* ATCC 29473, NCIMB 2194 *Carnobacterium maltaromaticum* (syn *C. piscicola*) ATCC 35586, NCIMB 2264 *Lactococcus garviae* (NCIMB 702927) (Stored frozen at less than –70°C using BS EN 12353:2006 recommended methods or cryopreservative beads)			
EN1656:2019 5.4.1.3	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			
EN1656:2019 5.4.1.4a	Cell suspensions prepared to between 1.5 x 10^8 and 5 x 10^8 cfu/ml			
EN1656:2019 5.4.1.4a	Prepared suspensions maintained in water bath at test temperature ± 1°C and used within 2 hours			
EN1656:2019 5.4.1.4b and modification 2	Dilutions prepared to 10^{-6} and 10^{-7} for counting and pour plates incubated at 22°C ± 1°C			
EN1656:2019 5.4.1.5 and modification 6	Prepare validation suspension (*Nv*) of ($3x10^2$) to ($3x10^3$) cfu/ml (approximately one fourth (1+3) of the 10^{-5} dilution of main bacterial suspension)			

EN1656:2019 5.4.1.5	For counting prepare validation suspension (*Nv*), 10^{-1} dilution with the diluent. Take 1ml of this mixture in duplicate and transfer into Petri dishes. Quickly add 12-15ml melted TSA cooled to 45°C ± 1°C			
EN1656:2019 5.4.1.5 and modification 2 and 5	Incubate at 22°C ± 1°C for 40-48hrs (10^{-2} dilution may also be prepared)			
Culture media and reagents:				
EN1656:2019 5.2.2.1	Reagents appropriate for microbiological purposes			
EN1656:2019 5.2.2.2	Water, either glass distilled or deionised water with conductivity of not greater than 2.1μS.cm^{-1}			
EN1656:2019 5.2.2.3	Media used for maintenance and viable counts is Tryptone Soya Agar			
EN1656:2019 5.2.2.4	Diluent used is Tryptone Sodium Chloride solution			
EN1656:2019 5.2.2.5	Neutralizer validated in accordance with 5.5.1.2, 5.5.1.3 and 5.5.2			
EN1656:2019 5.2.2.7	Standardized hard water freshly prepared and used within 12 hours			
Product test solutions:				
EN1656:2019 5.4.2	Concentration of product test solution must be 1.25 times the desired test concentration			
EN1656:2019 5.4.2	Product test solutions prepared freshly in standardized hard water and used within 120 min			
Experimental conditions:				
EN 1656:2019 5.5.1.1a and modification 3a	Contact temperature to be tested is 4°C ± 1°C			
EN 1656:2019 5.5.1.1a and modification 4a	Optional additional performance data at 20°C ± 1°C may be reported			
EN 1656:2019 5.5.1.1b and modification 3b	Contact time to be tested is 30 min ± 30 sec			
EN 1656:2019 5.5.1.1b and modification 4b	Optional data for 5 min ± 30 sec contact time may be reported			
EN 1656:2019 5.5.1.1a and modification 3a	Test suspension maintained at 4°C ± 1°C			
EN1656:2019 5.5.1.1c and modification 3c	Interfering substance as for high level soiling (10g/L yeast extract+10g/L bovine albumin) used for all dilutions			
Dilution-neutralization method:				
EN1656:2019 5.5.1.4	Equilibrate water and neutraliser to 20°C ± 1°C and all other reagents to test temperature			

EN1656:2019 5.5.2.2	Avoid touching upper part of test tube sides when adding bacteria			
EN1656:2019 5.5.2.2	Pipette 1ml interfering substance and 1ml bacterial test suspension into test tube			
EN1656:2019 5.5.2.2	Immediately start stopwatch, mix and place in water bath at test temperature eg 4°C ± 1°C			
EN1656:2019 5.5.2.2	After 2 min ± 30 sec add 8ml of product test solution			
EN1656:2019 5.5.2.2	Restart stopwatch, mix and return to water bath for contact time eg 30 mins ± 30 sec			
EN1656:2019 5.5.2.2	Mix just before end of contact time			
EN1656:2019 5.5.2.2	At end of contact time pipette 1ml test mixture into tube containing 8ml neutralizer and 1ml water in a water bath at 20°C ± 1°C			
EN1656:2019 5.5.2.2	After 5 min ± 30 sec transfer 2 x 1ml neutralized mixture into Petri dishes			
EN1656:2019 5.5.2.2	Quickly add 12-15ml melted TSA cooled to 45°C ± 1°C (in exceptional cases neutralizer can be added to TSA)			
EN1656:2019 5.5.2.6 and modification 2 and 5	Incubate at 22°C ± 1°C for 40-48 hours			
EN1656:2019 5.5.2.6	Count only plates showing well separated colonies			
EN1656:2019 5.5.2.6b	Using highest number of colonies note for each plate the exact number of colonies but record > 330 for any counts higher than 330 and determine the *Vc* values according to 5.6.2.2. All counts to be between 14 and 330 cfu/ml for plates			
EN1656:2019 5.5.2.6c	Calculate the numbers of cfu/ml in the test mixture *Na* and in the validation mixtures *A*, *B* and *C* using the method given in 5.6.2.4 and 5.6.2.6. Verify according to 5.7			

Experimental conditions validation and/or verification of the absence of any lethal effect in the test conditions:				
EN1656:2019 5.5.2.3a	1ml of the interfering substance plus 1ml of the validation suspension mixed and placed in waterbath at test temperature for 2min ± 30 sec			
EN1656:2019 5.5.2.3a	Add 8ml of hard water, mix and place in waterbath at test temperature for test contact time ± 30 sec			
EN1656:2019 5.5.2.3b	Take 1ml of this mixture in duplicate and transfer into Petri dishes. Quickly add 12-15ml melted TSA cooled to 45°C ± 1°C (ref 5.5.2.2b)			
EN1656:2019 5.5.2.3b and modification 2 and 5	Incubate at 22°C ± 1°C for 40-48hrs			
Neutralization control - verification of the absence of the toxicity of the neutralizer:				
EN1656:2019 5.5.2.4a	8ml of test neutralizer, and 1ml of water and 1ml of validation suspension mixed and placed in waterbath at 20°C ± 1°C for 5min ± 30 sec. Mix just before the end of this time			
EN1656:2019 5.5.2.4b	Take 1ml of this mixture in duplicate and transfer into Petri dishes. Quickly add 12-15ml melted TSA cooled to 45°C ± 1°C (ref 5.5.2.2b)			
EN1656:2019 5.5.2.3b and modification 2 and 5	Incubate at 22°C ± 1°C for 40-48hrs			
Dilution-neutralisation method validation:				
EN1656:2019 5.5.2.5	Validation of dilution-neutralization method - done for each test strain according to EN1656:2019 5.5.2.5			
EN1656:2019 5.5.2.5	1ml interfering substance, 1ml diluent and 8ml strongest product dilution placed in sterile tube and transferred to water bath at test temperature, eg 4°C ± 1°C, for contact time eg 30min ± 30 sec			
EN1656:2019 5.5.2.5	1ml of the mixture placed into tube containing 8ml neutralizer in water bath at 20°C and left for 5 min ± 30 sec			
EN1656:2019 5.5.2.5	Add 1ml of prepared validation suspension (*Nv*) and immediately start stopwatch			
EN1656:2019 5.5.2.5	Vortex and return to 20°C water bath for 30min ± 30 sec			
EN1656:2019 5.5.2.5	Vortex immediately before end of contact time			

EN1656:2019 5.5.2.5	Take 1ml of this mixture in duplicate and transfer into Petri dishes. Quickly add 12-15ml melted TSA cooled to 45°C ± 1°C			
EN1656:2019 5.5.2.6 and modification 2 and 5	Incubate at 22°C ± 1°C for 40-48 hours			
Calculation and expression of results:				
EN1656:2019 5.5.2.6b	Using highest number of colonies note for each plate the exact number of colonies but record > 330 for any counts higher than 330 and determine the *Vc* values according to 5.6.2.2, all counts to be between 14 and 330 cfu/ ml for plates			
EN1656:2019 5.7.3	Basic limits For each test organism check that: a) *N* is between $1.5x10^8$ and $5.0x10^8$ ($8.17 \leq \lg N \leq 8.70$), N_0 is between $1.5x10^7$ and $5.0x10^7$ ($7.17 \leq \lg N_0 \leq 7.70$) b) Nv0 is between 30 and 160(3.0x101 and 1.6x102) (*Nv* is between $3.0x10^2$ and $3.0x10^3$) c) A, B, C are equal to or greater than 0.5 x Nv_0 d) Control of weighted mean counts (5.7.2): quotient is not lower than 5 and not higher than 15 Explanation of terms and abbreviations (Section 5.6.1) where: *N* and *Nv* represent the bacterial suspensions, *Na* represents the bactericidal test mixture, *A* (experimental conditions control), *B* (neutralizer or filtration control), *C* (method validation) represent the different control test mixtures *N*, *Nv*, N_0, Nv_0, *Na* and *A*, *B* and *C* represent the number of cells counted per ml in the different test mixtures in accordance with Table 1 Section 5.6.1.1			
EN1656:2019 5.8.1	For each organism and product test concentration reduction in viability calculated using formula given in EN1656 5.8.1			

Conclusion and test report:				
EN1656:2019 5.9.2	The product is deemed to have passed the test if it demonstrates a 5 $\log_{10}$ or more reduction in viability within 30 min or less using the specified organisms and test conditions			
EN1656:2019 5.10	The test report shall refer to the EN1656 standard and shall include the information specified at EN1656 5.10 (also 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)
Print name:

Date:

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 14675:2015, (Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in veterinary area) 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: 2015 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) 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). See 5.2.2.4.3.
3. Optional data (Section 5.5.1):
    a. The product performance at 20 °C ± 1 °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.

* 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 for all times in the standard will not be regarded as an invalidation of the reported test results. Deviations ± > 30 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 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.

4. MDBK cells, used to multiply the virus (Section 5.5.2), should be replaced by Chinook salmon embryo (CHSE-214, ATCC CRL 1681) cells or alternative cell lines dependent on the virus to be tested. It is accepted that cell culture and preparation will differ from those specified in the standard to suit the cell lines used for this modified test.
5. Cells may be attached to the plate as a confluent monolayer of cells rather than in suspension (Sections 5.5.3 & 5.7.2). If this is the case cell culture techniques may differ.
6. Section 5.6: Virus dilutions and titrations may be made directly on microtitre plates containing a confluent (> 90%) cell monolayer and culture medium by serial 1 in 10 dilutions. To do this, virus suspension or test mixture is added to each well of the first row and diluted down the plate. When using this method, the neutralising dilutions will not be made in cold diluent or transferred to 4°C/crushed ice as stated in the standard (Section 5.7.2). When using this modification, it is accepted that the media will contain 10% foetal bovine serum (FBS).
7. Titrations may be made with ≥ 6 wells per dilution (Standard states 8 wells per dilution section 5.6 and 5.7.2).
8. Modification to Annex B1(b): Treatment of cells is with test mixture containing the lowest apparently non-cytotoxic dilution of the product compared to test mixture where product is replaced with water (rather than comparison with MEM +2% FCS).
9. Modification to Section 5.8.1: Dilute the product 1/10 then add the virus for 30 min ± 10* sec at 4 °C ± 1 °C before titration (rather than dilution of the prepared test mixture, as stated in the standard). The difference of titre with the test suspension shall be <1 log, (not <0.5 log as stated in 5.8.1 and 5.8.2).
10. The test virus reference inactivation test using formalin (Annex B.2) is optional.
11. The level of cytopathic effect (CPE) recorded (section 6.1) is not necessary, only record “+” if CPE is present or “–” if no CPE.
12. Replace 37 °C with 15°C ± 1°C throughout (temperature for incubating CHSE-214 cells).
13. 5.3.2.11 Suggest 6-well multiplates are used here instead of Petri dishes.
14. 5.6.4 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. 10% neutral buffered formalin can be used for fixation.
15. Annex B1a. Virus is replaced with medium not water.

If any problems arise following this standard (e.g. cell cytotoxicity), the Cefas Disinfectant Listing 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 Disinfectant 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 of 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 14675:2015 Section 7.2 and Annex D. The results should include details of all relevant test results, including testing for cytotoxicity caused by product solutions (Annex B.1) and results of titration of test virus suspension (Annex B.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 recommend ed 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 (Modification 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: 2015 Section 7.2 and Annex D), 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, 2015. BS EN 14675:2015 Chemical disinfectants and antiseptics- Quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in veterinary area- Test method and requirements (Phase 2 step 1). BSi British Standards, London UK, http://www.bsi-global.com/en/

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, http://www.bsi-global.com/en/

Verner - Jeffreys DW, Ridout N, Joiner C, Reese RA, Husby A, Dixon PF. (2019) Development of virucidal and bactericidal aquaculture disinfectant testing standards. Aquaculture 286, 190-197.

Appendix 4 - Example Modified EN14675:2015 Quality Assurance Checklist

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			
Legal status	GB Biocidal Products Regulation (BPR) compliant			
Expiry date	Listed in test report			
Storage	Listed in test report			
EN 14675:2015 5.4	Concentration of the product test solution shall be 1.25 times the desired test concentration			
EN 14675:2015 5.4	Product test solutions prepared freshly in hard water (water for ready to use product) and used within 120 mins			
Experimental conditions:				
EN 14675:2015 5.5.1a and modification 2a	Contact temperature to be tested is 4°C ± 1°C			
EN 14675:2015 5.5.1a and modification 3a	Optional additional performance data at 20°C ± 1°C may be reported			
EN 14675:2015 5.5.1b and modification 2b	Contact time to be tested is 30 min ± 30sec			
EN 14675:2015 5.5.1b and modification 3b	Optional data for 5 min ± 30 sec contact time may be reported			
EN 14675:2015 5.5.1c and modification 2c	Interfering substance as for high level soiling (10g/L bovine albumin plus 10g/L yeast extract) used for all dilutions			
EN 14675:2015 5.5.1d and modification 1	Test agent IPNV A2 Spjarup			
EN 14675:2015	Additional test agent listed in test report			
EN 14675:2015 5.2.2.2	Water (for culture media); freshly glass distilled and not de-mineralised			
EN 14675:2015 5.2.2.3	Hard water (for dilution of product); reagents used are of analytical grade and/or appropriate for virological purposes			
EN 14675:2015 5.2.2	Cell culture medium; any medium demonstrably suitable for culture of CHSE214 cells (eg L-15+10% foetal bovine serum (FBS))			

EN 14675:2015 5.5.3	Preparation of cell line; cell suspensions prepared by trypsinising (eg 0.25% trypsin (Ethylenediaminetetraacetic acid (EDTA)) solution)			
EN 14675:2015 5.5.3	Preparation of cell line on microtitre plates; cells split at sufficient ratio in cell culture medium +10 % FBS to enable formation of monolayer in at least two days			
EN 14675:2015 5.6 and modification 6	Viral suspension dilutions/titration; ≥ 6 wells per dilution if titrated directly in plate			
Cytotoxicity:				
EN 14675:2015 Annex B.1(a) and modification 15	Solutions prepared as for the test using medium instead of virus and including interfering substance, then inoculated into monolayer cell cultures			
EN 14675:2015 Annex B.1(b)	Comparative virus titrations performed on cells that have and have not been treated with disinfectants			
EN 14675:2015 and modification 11	Record +ve results as '+' and wells without CPE as '-'			
EN 14675:2015 Annex B.1(a)	Only dilutions showing a low degree of cell destruction (<25% of monolayer) used for determination of residual infectivity			
EN 14675:2015 6.4 d) and Annex B1(b) and modification 8	Comparative virus titration on cells inoculated with test mixture or test mixture where product is replaced with water result in a difference of $\log_{10}$ < 1.0 of virus titre			
EN 14675:2015 6.4 c) and Annex B1(a) and (b)	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			
Product neutralisation:				
EN 14675:2015 5.8.1 and modification 9	Prepare the test mixture (section 5.4 and 5.7.2): 8ml product, 1ml interfering substance, 1ml medium. Dilute the test mixture 1 in 10, achieved by adding 1ml test mixture to 8ml medium and then adding 1ml stock virus. If required, further dilute to 1 in 100, achieved by adding 0.1ml test mixture to 8.9ml medium and then adding 1ml stock virus. Incubate for 30 min ± 30 sec at 4°C ± 1°C before titration.			
EN 14675:2015 and modification 9	1) At 1 in 10 dilution of product, virus is ± 1 $\log_{10}$ of the control (control = water instead of product)			If no, see 2
EN 14675:2015 and modification 9	2) At 1 in 100 dilution of product, virus is ± 1 $\log_{10}$ of the control (control = water instead of product)			If no, see 3

EN 14675:2015 and modification 9	3) If not achieved using 1 in 100 dilution of product, test for alternative neutralisation method to achieve virus titre of ± 1 $\log_{10}$ of the control			
Reference inactivation test (optional):				
EN 14675:2015 6.4 b) and Annex B3 This test is optional - see modification 10	The difference of the logarithmic titre of the virus control minus the logarithmic titre of the virus in the reference inactivation test is between -0.5 and -2.5 after contact time eg 30 min ± 30 sec			
Test method:				
EN 14675:2015 5.7.2	Vortex just before end of contact time			
EN 14675:2015 5.7.2 and modification 2b and 6	0.5ml test mixture taken after contact time eg 30 min ± 30 sec			
EN 14675:2015 5.7.2 and modification 2a and 6	1) Add 0.5ml of test mixture to 4.5ml minimum essential medium (MEM) + 2% FBS at test temperature eg 4 ± 1°C			If yes, see 2 If no, see 4
EN 14675:2015 5.7.2 and modification 2a and 6	2) Prepare dilutions to 10^{-8} in MEM + 2% FBS and keep at test temperature eg 4 ± 1°C			If yes, see 3 If no, see 4
EN 14675:2015 5.7.2 and modification 7	3) ≥ 6 wells of confluent monolayer of cells in micro titre plates inoculated with each dilution of test mixture			If yes, see 6
EN 14675:2015 5.7.2 and modification 6	4) ≥ 6 wells of confluent monolayer of cells in micro titre plates inoculated with the 1 in 10 dilution of test mixture			If yes, see 5
EN 14675:2015 5.7.2 and modification 6	5) Gently mix the test mixture in wells of micro titre plate by aspirating/dispensing the mixture, taking care not to disturb the cell monolayer. Make serial 1 in 10 dilutions on the plate down to 10^{-8}. Remove sufficient medium from the final dilution such that all wells on the micro titre plate contain the same volume of medium			If yes, see 6
EN 14675:2015 5.7.2	6) Titre of infectivity calculated after incubation by formula described by Spearman and Kärber (Annex C.1)			
EN 14675:2015 6.4 a)	A product passes the test if ≥ 4 $\log_{10}$ reduction is achieved after treatment			
EN 14675:2015 7.1	Product deemed to have passed if demonstrates ≥ 4 $\log_{10}$ reduction in titre within the test contact time eg 30 min ± 30 sec, at test temperature, eg 4°C ± 1°C, under the defined test conditions			

Reporting of results:				
EN 14675:2015 6.6	Results tabulated as raw data and also expressed as negative logarithmic values of 50% tissue culture infectious dose ($TCID_{50}$)			
EN 14675:2015 6.6	If no virus multiplication is observed in the highest concentration, this value is denoted with the sign ≤ If there is virus multiplication observed in all dilutions, result denoted with the sign ≥			
The report shall include:				
EN 14675:2015 7.2	The test report should refer to the EN14675:2015 standard and shall include the information specified in EN14675:2015 Section 7.2 (see appendix 6 of this form) and shall also include:- • Dilution to stop action of product			

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)
Print name:

Date:

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 1656:2019 Section 5.10)

Determination of bactericidal activity in high soiling conditions.

a. Identification of the testing 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. Date(s) of test (period of analysis):

   ii. Diluent used for product test solution (hard water or distilled water):

   iii. Product test concentrations (= desired test concentrations according to 5.4.2):

   iv. Appearance of the product dilutions:

   v. Contact time(s):

   vi. Test temperature(s):

   vii. Interfering substance:

   viii. Stability and appearance of the mixture during the procedure (note the formation of any precipitate or flocculant):

   ix. Temperature of incubation:

   x. Neutralizer or rinsing liquid:

   xi. Identification of bacterial strains used:

e. Test results, to include controls and validation, evaluation of bactericidal activity and number of replicates per test organism:

   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: Print Name: Date:

Appendix 6 – Model Test Report Format for Standard EN14675 (reference European Standard EN 14675:2015 Section 7.2)

Determination of 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 their concentration(s) (optional):

   ix. Appearance of product:

c. Experimental conditions:

   i. Dates of test (period of analysis):

   ii. Diluent used for product test solution (hard water or distilled water):

   iii. Product test concentrations (= desired test concentrations according to 5.4):

   iv. Appearance of product dilutions:

   v. Contact time(s):

   vi. Test temperature(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:

   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: