Verbund für Angewandte Hygiene e.V.
Desinfektionsmittel-Kommission

Requirements and Methods for VAH Certification of Chemical Disinfection Procedures

Edited by: VAH Disinfectants Commission

Excerpt: Requirements for the virucidal activity

- **Chapter 4.2: Efficacy testing against viruses**
- **Annex V (Requirements for the virucidal activity)**

As of 1 September 2023

Overview of changes made on updating the requirements for the virucidal activity

As of 1 September 2023

Chapter 4

In addition to minor formal changes, the following passages in Chapter 4.2 were fundamentally revised:

- For efficacy testing against viruses, the activity spectrum “virucidal activity PLUS” has been introduced for surface disinfection (see Chapter 4, page 2f).

Annex V1-V4

In addition to minor formal changes, the following passages in Annex V1-V4 were fundamentally revised:

- For hygienic hand disinfection (V1A), surface disinfection (V2A), instrument disinfection (V3A) and chemothermal laundry disinfection (V4A), it is recommended to test the concentration-time relations specified in the respective Table V. Other concentration-time relations – **but at least three** – are possible, provided that the kinetics and ineffective limit values are evident from these. A reference test and cytotoxicity test must be performed for each test run. If detoxification of the test batch is done with columns, the same test run must be carried out additionally in parallel without columns.
- For surface disinfection a certificate shall only be issued if the requirements in the suspension test **and** in the simulated-use (practical) test have been met, i.e. certification is not possible on the basis of suspension tests alone. The bactericidal and yeasticidal activity confirmed by VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification (see Chapter V1A, page 4).
- To take account of the interactions seen in a 4-field test between the wipe and the disinfectant used when testing the virucidal activity, the liquid expressed aseptically from the wipes is used as disinfectant. If no specific wipes have been prescribed, the standard wipe used in the 4-field test is impregnated with disinfectant and the disinfectant is then expressed after 5 min (see Chapter V2A, page 7).
- If the product is to be used for surface disinfection in the form of a spray with mechanical action, testing must be performed with a solution of the collapsed spray (see Chapter V2A, page 7).
- For instrument disinfection a certificate shall only be issued if the requirements in the suspension test **and** in the simulated-use test have been met, i.e. certification is not possible on the basis of suspension tests alone (see Chapter V3A, page 8).
- For chemothermal textile disinfection, Table V4.2 *Test Conditions* (test batch and control) has been added.

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4 Efficacy against specific pathogens

4.2 Efficacy testing against viruses

The inclusion of virucidal properties in the VAH List is intended as a means of giving the user the opportunity to select disinfectants for which, based on the current state of knowledge, the activity claims “active against enveloped viruses”, “limited spectrum of virucidal activity”, “virucidal activity”, and for surface disinfectants additionally “virucidal activity PLUS”, has been demonstrated in the quantitative suspension test and in the test under simulated-use conditions (if available).

On receipt of a corresponding application from a company for certification (of a product), the test reports and expert opinions relating to the respective products will be reviewed by independent experts.

For the conformity assessment procedure two independent expert opinions regarding the respective activity spectrum, including test reports, confirming efficacy in the claimed concentration-time relation must be submitted.

The test methods with the associated test viruses, interfering substances and test conditions are specified for the respective fields of application.

Furthermore, for tests based on a European standard the effective concentration-time relation must be confirmed in a second independent test. The reproducibility test (second test batch) must be performed using at least the use concentration and, as controls, must include the virus control, the cytotoxicity test and the reference test.

Since European standards and the corresponding DVV guidelines may be used as test methods, the test report must clearly state which method was used for testing. To improve interpretation of the results all deviations from the respective method must be clearly described. If a large volume-plating method is used, the respective dilutions, volumes and number of microtitre plates used for all test and control batches must also be given.

The bactericidal and yeasticidal (levoricidal) activity confirmed by the VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification.

The contact time for activity against viruses featured in the VAH List must not be shorter than that specified for the bactericidal/yeasticidal efficacy. The test concentrations and contact times must be selected such that the relationship between the disinfectant virucidal activity and the concentration and contact time is evident from the test results (kinetics).

The following activity claims may be certified and listed: “active against enveloped viruses”, “limited spectrum of virucidal activity”, “virucidal activity”, and for surface disinfectants additionally “virucidal activity PLUS” [3,4]:

- **Active against enveloped viruses**
- **Limited spectrum of virucidal activity** – effective against enveloped viruses and adenoviruses, norovirus and rotavirus
- **Virucidal activity** – effective against enveloped and non-enveloped viruses (see examples in **Table 4.1**) except *Parvoviridae*, HAV, HEV
- **Virucidal activity PLUS** – effective against enveloped and non-enveloped viruses, including adeno-associated vector viruses, *Parvoviridae*, hepatitis A virus and hepatitis E virus [4].

Table 4.1 gives a summary of the test viruses used and of the viruses thus covered.

Table 4.1: Test viruses used for efficacy testing of disinfectants and selected viruses covered by the test viruses [3,4,5].

Activity spectrum	Test viruses	Viruses covered by the activity spectrum (examples)
Active against enveloped viruses	**– Vaccinia virus (strain Elstree or MVA)** **– BVDV* (Bovine Viral Diarrhoea Virus)** *Surrogate virus for Hepatitis C Virus, only used for products with oxidative activity	**Bloodborne viruses** – Hepatitis B virus (HBV) – Hepatitis C virus (HCV) – Human immunodeficiency virus (HIV) **Viruses causing respiratory infections** – Human coronaviruses (HCoV) 229E, HKU1, NL63 and OC43, SARS-CoV-2, MERS-CoV – Influenza virus A (e.g. H1N1, H3N2) and B – Metapneumovirus – Respiratory syncytial virus (RSV) **Viruses with other transmission routes** – Ebola virus, hantavirus, Lassa virus, Marburg virus – Rabies virus **Herpesviridae** – Cytomegalovirus (CVM) – Epstein Barr virus (EBV) – Herpes simplex viruses type 1 and 2 (HSV-1, HSV-2) – Varicella Zoster virus (VZV) **Orthopoxviridae** – mpox virus **Other viruses causing vaccine-preventable diseases** – Measles virus – Mumps virus – Rubella virus **Vector-borne viruses** – Bunyavirus (causing sandfly fever) – Crimean-Congo haemorrhagic fever virus – Dengue virus – Tick-borne encephalitis virus (TBEV) – West Nile virus (causing West Nile fever) – Yellow fever virus
Limited spectrum of virucidal activity	**– Adenovirus (type 5, Adenoid 75 strain)** **– Murine norovirus (MNV, S99 Berlin strain)**	**Viruses covered by limited spectrum of virucidal activity and additionally the following lipophilic non-enveloped viruses:** **Viruses causing gastrointestinal infections** – Adenovirus serotype 40 and 41 – Norovirus – Rotavirus **Viruses causing respiratory infections** – Adenovirus serotype 7 **Viruses causing keratoconjunctivitis** – Adenovirus serotype 8, 19 and 37

(continued overleaf)

Table 4.1 (continuation**):** Test viruses used for efficacy testing of disinfectants and selected viruses covered by the test viruses [3,4,5].

Activity spectrum	Test viruses	Viruses covered by the activity spectrum (examples)
Virucidal activity	**– Adenovirus (type 5, Adenoid 75 strain)** **– Poliovirus (type 1, LSc-2ab strain)** **– Polyomavirus SV40 (simian virus 40, strain 777)** **– Murine norovirus (MNV, S99 Berlin strain)** **Only applicabel for chemothermal processes >30 °C (textiles) or >40 °C (instruments):** **– Murine parvovirus (minute virus of mice (MVM), rodent protoparvovirus 1)**	**Viruses covered by limited spectrum of virucidal activity and additionally:** *Papillomaviridae* *Picornaviridae* – Enteroviruses: coxsackieviruses, echoviruses, polioviruses, EV 71, – Parechoviruses: echovirus 11, 22 and 23 **When using chemothermal disinfection procedures (textiles > 30 °C or instruments > 40 °C), the following viruses are also covered by virucidal activity spectrum:** – *Parvoviridae* – Adeno-associated viruses (AAV) – Bocavirus – Parvovirus B19
Virucidal activity PLUS *(this activity spectrum can only be claimed for surface disinfection)*	**– Murine parvovirus (minute virus of mice (MVM), rodent protoparvovirus 1)**	**Viruses covered by virucidal activity spectrum and additionally:** Hepatitis A virus Hepatitis E virus *Parvoviridae* – Adeno-associated viruses (AAV) – Bocavirus – Parvovirus B19

Annex V (virucidal activity) sets out in detail the requirements to be met for certification of virucidal procedures.

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ANNEX V (Requirements for the virucidal activity)

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These requirements for virucidal efficacy were developed by members of the Virucidal Efficacy Working Group of the VAH Disinfectants Commission on a voluntary basis and without the influence of commercial interest groups, and were agreed upon in consensus with the Disinfectants Commission and released for publication.

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ANNEX V: Virucidal activity
V1A Hygienic hand disinfection

Requirements

For the conformity assessment procedure two independent expert opinions (assessments), including test reports, confirming the efficacy in the claimed concentration-time relation must be submitted.

To that effect, expert opinions compiled in accordance with the DVV/RKI Guideline 2008 [1] or 2015 [2] may be submitted. Expert opinions based on DVV/RKI Guideline 2005 [3] may need to be supplemented with additional tests in order to comply with the requirements of DVV/RKI Guideline 2015 or 2008 [1,2]. Alternatively, expert opinions based on DIN EN 14476 [4] may also be submitted.

Furthermore, for tests based on a European standard the use concentration must be confirmed in the respective test report in a second independent test batch and, as controls, must include the virus control, cytotoxicity test and reference test. The mean confidence interval for two independent tests must each be ≤ 0.5 lg.

The bactericidal and yeasticidal activity confirmed by the VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification. This also includes activity within the claimed concentration and contact time (exposure time) in the simulated-use test with *E. coli* based on Method 11 or DIN EN 1500 [5].

The concentration-time relation for activity against viruses featured in the VAH List must not be shorter than that specified for the VAH-listed bactericidal and yeasticidal efficacy. The test concentrations and contact times must be selected such that the relationship between the disinfectant virucidal activity and the concentration and contact time is evident from the test results (kinetics). It is recommended to test the concentration-time relations specified in **Table V1.1**. Other concentration-time relations – but at least three – are possible, provided that the kinetics and ineffective values are evident from these.

Obligatory:

- *Determination of the efficacy claim 'active against enveloped viruses' in the quantitative suspension test (method based on DVV/RKI 2008 or 2015 [1, 2] or DIN EN 14476 [4]*

The test product must reduce the virus titre of the test viruses listed in **Table V1.1** under the specified conditions within the stipulated contact time(s) at 20 °C by at least 4 lg levels.

Optional:

– *Determination of the efficacy claims limited spectrum of virucidal activity and/or virucidal activity in the quantitative suspension test (method based on DVV/RKI 2008 or 2015 [1,2] or DIN EN 14476 [4]*

The test product must reduce the virus titre of the test viruses listed in **Table V1.1** under the specified conditions within the stipulated contact time(s) at 20 °C by at least 4 lg levels.

A reference and cytotoxicity test must be performed for each test run. If detoxification of the test batch is done with columns, the same test run must be carried out additionally in parallel without columns [see 2, Annex 7].

For disinfectants declared as having limited spectrum of virucidal activity or virucidal activity it is assumed that, because of their activity against the non-enveloped test viruses, efficacy against vaccinia virus is also covered. For new active ingredients/substances and/or mechanisms of action, this may have to be verified additionally.

Table V1.1: Test conditions in the quantitative suspension test.

Activity spectrum	Test organisms	Test method	Interfering substance[1]/ Test concentrations[2]	Test temperature [°C]	Contact times[3]
Active against enveloped viruses	Vaccinia virus BVDV[4]	DVV/RKI [1 and 2] or DIN EN 14476 [4][5]	DVV/RKI dirty or clean conditions (EN) / undiluted	20 ± 1	15 s, 30 s, 1 min
Limited spectrum of virucidal activity	Adenovirus Norovirus	DVV/RKI [1, 2 and 3] or DIN EN 14476 [4][5]	RKI/DVV dirty or clean conditions (EN) / undiluted	20 ± 1	15 s, 30 s, 1 min, 1.5 min, 2 min
Virucidal activity	Poliovirus Adenovirus Norovirus SV40	DVV/RKI [1, 2 and 3] or DIN EN 14476 [4][5]	RKI/DVV dirty or clean conditions (EN) / undiluted	20 ± 1	15 s, 30 s, 1 min, 1.5 min, 2 min

[1]The interfering substances based on DVV/RKI are 10 % FCS (foetal calf serum) and distilled water or based on DIN EN 14476 clean conditions (low organic challenge) consisting of 0.3 % BSA (bovine serum albumin).
[2]To record the efficacy limit at least two concentrations (use concentration and an ineffective concentration) must be tested.
[3]Three of the listed contact times shall be tested, including the claimed contact times. The contact times shall be between 15 s and 2 min. A maximum contact time of 60 s shall be aspired to.
[4]Additionally for products with oxidative activity
[5]Test reports based on EN 14476:2013 continue to be valid.

If available, test reports based on prDIN EN 17430 (simulated use-test with the murine norovirus) may also be submitted for the virucidal efficacy (*limited spectrum of virucidal activity and/or virucidal activity*) [6].

To compare the results of the test procedure and reference procedure and evaluate the test procedure, the following requirements must be met:

- Exploitable results must be available for at least 18 subjects.
- The total mean value of the prevalues for the reference product and test product must be at least 4 lg.

- For the reference product no more than three individual lg reductions may be < 2 and the absolute difference of the mean differences between the lg reductions of the reference product group → test product and test product group → reference product must be less than 2.
- The test product must not be inferior to the reference product (Hodges-Lehmann) p = 0.025.
- The limit for inferiority = 0.35 lg units.

Test viruses as per DVV/RKI guidelines and DIN EN 14476

Adenovirus	=	Adenovirus type 5, Adenoid 75 strain
BVDV	=	Bovine Viral Diarrhoea Virus, NADL strain
Norovirus	=	Murine norovirus, S99 Berlin (MNV) strain
Poliovirus	=	Poliovirus vaccination type 1 strain, LSc-2ab strain
SV40	=	Polyomavirus (SV 40), 777 strain
Vaccinia virus	=	Modified vaccinia virus Ankara (MVA) or vaccinia virus, Elstree strain

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Note: VAH certificates based on the prEN version continue to be valid.

V2A Surface disinfection

Requirements

For the conformity assessment procedure two independent expert opinions, including test reports, confirming the efficacy in the claimed concentration-time relation in quantitative suspension tests and simulated-use tests must be submitted.

To that effect, expert opinions compiled in accordance with DVV/RKI Guideline 2008 [1] or 2015 [2] may be submitted. Expert opinions based on DVV/RKI Guideline 2005 [3] may need to be supplemented with additional tests in order to comply with the requirements of DVV/RKI Guideline 2015 or 2008 [1,2]. Alternatively, expert opinions based on DIN EN 14476 [4] may also be submitted.

The simulated-use surface tests must be performed in accordance with the DVV Guideline 2012 [5] or EN 16777 [6] (surface disinfection without mechanical action). This applies also to products with mechanical action for as long as no simulated-use test methods with mechanical action have been published for surface disinfection. To take account of the interactions seen in a 4-field test between the wipe and the disinfectant used for testing the virucidal activity, the liquid expressed aseptically from the wipes is used as disinfectant. If no specific wipes have been prescribed, the standard wipe used in the 4-field test is impregnated with disinfectant and the disinfectant is then expressed after 5 min. Disinfectant must be expressed from as many wipes as needed to collect enough liquid in a sterile container. The number of wipes from which disinfectant has been expressed and the amount of liquid expressed [ml] must be recorded.

If the product is to be used in the form of a spray with mechanical action, testing must be performed with a solution of the collapsed spray. Using as many pump actuations as needed, collect the foam in a sterile measuring cylinder and wait until a sufficient amount of liquid has accumulated to carry out testing. In the test report record the number of pump actuations, the amount of foam produced [ml] and the amount of liquid collected [ml].

Furthermore, for tests based on a European standard the use concentration must be confirmed in the respective test report in a second independent test batch and, as controls, must include the virus control, cytotoxicity test and reference test. The mean confidence interval for two independent tests must each be ≤ 0.5 lg.

A certificate shall only be issued if the requirements in the suspension test **and** in the simulated-use test have been met, i.e. certification is not possible on the basis of suspension tests alone.

The bactericidal and yeasticidal (levoricidal) activity confirmed by the VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification.

The concentration-time relation for activity against viruses featured in the VAH List must not be shorter than that specified for the VAH-listed bactericidal and yeasticidal efficacy. The test concentrations and

contact times must be selected such that the relationship between the disinfectant virucidal activity and the concentration and contact time is evident from the test results (kinetics). It is recommended to test the concentration-time relations specified in **Table V2.1 and V2.2**. Other concentration-time relations – but at least three – are possible, provided that the kinetics and ineffective spectrum are evident from these.

Obligatory:

- *Determination of the virucidal efficacy (active against enveloped viruses, limited spectrum of virucidal activity, virucidal activity and/or virucidal activity PLUS) in the quantitative suspension test (method based on DVV/RKI 2008 or 2015 [1,2] or DIN EN 14476 [4].*
- *Determination of the virucidal efficacy (active against enveloped viruses*, *limited spectrum of virucidal activity*, *virucidal activity and/or virucidal activity* PLUS*)) in the simulated-use surface test (DVV Guideline 2012 [5] or EN 16777 [6]).*

The test product must reduce the virus titre of the test viruses listed in **Table V2.1** and **V2.2** under the specified conditions within the stipulated contact time(s) at the test temperature by at least 4 lg levels.

A reference and cytotoxicity test must be performed for each test run. If detoxification of the test batch is done with columns, the same test run must be carried out additionally in parallel without columns [see 2, Annex 7].

For disinfectants declared as having limited spectrum of virucidal activity, virucidal activity or virucidal activity PLUS it is assumed that, because of their activity against the non-enveloped test viruses, efficacy against vaccinia virus is also covered. For new active ingredients/substances and/or mechanisms of action, this may have to be verified additionally.

The following tables showing the simulated-use tests (**Table V2.2 and V2.3**) apply only in combination with quantitative suspension tests (**Table V2.1**).

Table V2.1: Test conditions in the quantitative suspension test.

Activity spectrum	Test organisms	Test method	Interfering substance[1]/ Test concentrations[2]	Test temperature [°C]	Contact times [min][3]
Active against enveloped viruses	Vaccinia virus BVDV[4]	*DVV/RKI [1, 2]* *or* *DIN* EN 14476 [4][5]	DVV/RKI or clean or dirty/ use concentration[2]	20 ± 1	1, 5, 15, 30, 60
Limited spectrum of virucidal activity	Adenovirus Norovirus	DVV/RKI [1, 2] or DIN EN 14476 [4][5]	DVV/RKI or clean or dirty/ use concentration[2]	20 ± 1	
Virucidal activity	Poliovirus Adenovirus Norovirus SV40	DVV/RKI [1, 2] or DIN EN 14476 [4][5]	DVV/RKI or clean or dirty/ use concentration[2]	20 ± 1	
Virucidal activity PLUS	Poliovirus Adenovirus Norovirus SV40	DVV/RKI [1, 2] or DIN EN 14476 [4][5]	DVV/RKI or clean or dirty/ use concentration[2]	20 ± 1	

[1]The interfering substances based on DVV/RKI are 10 % FCS (foetal calf serum) and distilled water. Based on DIN EN 14476, the test batch with a lower organic challlenge consisting of 0.3 % BSA (bovine serum albumin) is considered to be under clean conditions and the test batch with 3% BSA and 3% sheep erythrocytes is considered to be under dirty conditions.
[2]To record the efficacy limit at least two concentrations (use concentration and an ineffective concentration) must be tested (for gradations, see Chapter 5).
[3] Three of the listed contact times shall be tested, including the claimed contact times.
[4]Additionally for products with oxidative activity
[5]Test reports based on EN 14476:2013 continue to be valid.

Table V2.2: Test conditions in the simulated-use test.

Activity spectrum	Test organisms	Test method	Interfering substance[1]/ Test concentrations[2]	Test temperature [°C]	Contact times [min][3]
Active against enveloped viruses	Vaccinia virus	DVV Guideline 2012 [5] or EN 16777 [6]	Clean or dirty/ use concentration[2]	22 ± 3	1, 5, 15, 30, 60
Limited spectrum of virucidal activity	Adenovirus Norovirus	DVV Guideline 2012 [5] or EN 16777 [6]	Clean or dirty/ use concentration[2]	22 ± 3	
Virucidal activity	Adenovirus Norovirus	DVV Guideline 2012 [5] or EN 16777 [6]	Clean or dirty/ use concentration[2]	22 ± 3	
Virucidal activity PLUS	Adenovirus Norovirus Parvovirus	DVV Guideline 2012 [5] or EN 16777 [6]	Clean or dirty/ use concentration[2]	22 ± 3	

[1] The test batch consisting of 0.3 % BSA (bovine serum albumin) is considered to be under clean conditions and the test batch with 3% BSA and 3% sheep erythrocytes is considered to be under dirty conditions.
[2]To record the efficacy limit values at least two concentrations (use concentration and an ineffective concentration) must be tested (for gradations, see Chapter 5).
[3] The contact times to be tested are based on Table V2.3.

Table V2.3: The contact times to be selected in the individual test runs for simulated-use testing of surface disinfectants

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Claimed contact time	1st test run	2nd test run
1 min	0.5 min, 1 min	1 min
5 min	1 min, 5 min	5 min
15 min	5 min, 15 min	15 min
30 min	15 min, 30 min	30 min
60 min	30 min, 60 min	60 min

The simulated-use tests must be performed in all cases in two test runs:

1. Test run: In each case, at least two test surfaces per claimed concentration-time relation (see **Table V2.2** and **V2.3**), virus control (before and after drying), cytotoxicity test and reference test
2. Test run: In each case, at least two test surfaces per claimed concentration-time relation, virus control (before and after drying), cytotoxicity test and reference test

Test viruses as per DVV/RKI guidelines and DIN EN 14476 or DIN EN 16777

Adenovirus = Adenovirus type 5, Adenoid 75 strain

BVDV = Bovine Viral Diarrhoea Virus, NADL strain

Norovirus = Murine norovirus, S99 Berlin (MNV) strain

Parvovirus = Murine parvovirus (minute virus of mice, rodent protoparvovirus 1) (MVM)

Poliovirus = Poliovirus vaccination type 1 strain, LSc-2ab strain

SV40 = Polyomavirus (SV 40), 777 strain

Vaccinia virus = Modified vaccinia virus Ankara (MVA) or vaccinia virus, Elstree strain

References

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V3A Instrument disinfection (as immersion disinfection)

Requirements

For the conformity assessment procedure two independent expert opinions, including test reports, confirming the efficacy in the claimed concentration-time relation in quantitative suspension tests and simulated-use tests must be submitted.

To that effect, expert opinions compiled in accordance with DVV/RKI Guideline 2008 [1] or 2015 [2] may be submitted. Expert opinions based on DVV/RKI Guideline 2005 [3] may need to be supplemented with additional tests in order to comply with the requirements of DVV/RKI Guideline 2015 or 2008 [1,2]. Alternatively, expert opinions based on DIN EN 14476 [4] may also be submitted.

The simulated-use surface tests must be performed in accordance with EN 17111 [5]. Furthermore, for tests based on a European standard the use concentration must be confirmed in the respective test report in a second independent test batch and, as controls, must include the virus control, cytotoxicity test and reference test. The mean confidence interval for two independent tests must each be ≤ 0.5 lg.

A certificate shall only be issued if the requirements in the suspension test **and** in the simulated-use test have been met, i.e. certification is not possible on the basis of suspension tests alone.

The bactericidal and yeasticidal activity confirmed by the VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification.

The concentration-time relation for activity against viruses featured in the VAH List must not be shorter than that specified for the VAH-listed bactericidal and yeasticidal efficacy. The test concentrations and contact times must be selected such that the relationship between the disinfectant virucidal activity and the concentration and contact time is evident from the test results (kinetics). It is recommended to test the concentration-time relations specified in **Table V2.1 and V2.2**. Other concentration-time relations – but at least three – are possible, provided that the kinetics and ineffective values are evident from these.

Obligatory:

- *Determination of the virucidal efficacy (active against enveloped viruses and/or virucidal activity) in the quantitative suspension test (method based on DVV/RKI 2008 or 2015 [1,2] or DIN EN 14476 [4]*
- *Determination of the virucidal efficacy (active against enveloped viruses and/or virucidal activity) in the simulated-use test as per DIN EN 17111 [5].*

  The test product must reduce the virus titre of the test viruses listed in **Table V3.1 and V3.2** under the specified conditions within the stipulated contact time(s) at the test temperature by at least 4 lg levels.

A reference and cytotoxicity test must be performed for each test run. If detoxification of the test batch is done with columns, the same test run must be carried out additionally in parallel without columns [see 2, Annex 7].

For disinfectants declared as having limited spectrum of virucidal activity or virucidal activity it is assumed that, because of their activity against the non-enveloped test viruses, efficacy against vaccinia virus is also covered. For new active ingredients/substances and/or mechanisms of action, this may have to be verified additionally.

The following tables showing the simulated-use tests (**Table V3.2 and V3.3**) apply only in combination with quantitative suspension tests (**Table V3.1**)

Table V3.1: Test conditions in the quantitative suspension test.

Activity spectrum	**Test organisms**	**Test method**	**Interfering substance[1]/ Test concentrations[2]**	**Test temperature [°C]**	**Contact times [min][3]**
Active against enveloped viruses*	Vaccinia virus BVDV[4]	DVV/RKI [1, 2] or in accordance with DIN EN 14476 [4][5]	DVV/RKI or under clean or dirty conditions/ use concentration	20 ± 1	1, 5, 15, 30, 60
Virucidal instrument disinfection at < 40 °C	Poliovirus Adenovirus Norovirus SV40	DVV/RKI [1, 2] or DIN EN 14476 [4][5]	DVV/RKI or under clean or dirty conditions/ use concentration	20 ± 1 to < 40 ± 1	
Virucidal instrument disinfection at ≥ 40 °C	Parvovirus	DVV/RKI [1, 2] or DIN EN 14476 [4][5]	DVV/RKI or under clean or dirty conditions/ use concentration	≥ 40 ± 1 to ≤ 70 ± 1	

* For combined detergent/disinfectant precleaning products, suitable methods must be used to demonstrate the absence of protein-fixing properties (e.g. Amido black stain).

[1] The interfering substances based on DVV/RKI are 10 % FCS (foetal calf serum) and distilled water. Based on DIN EN 14476, the test with a lower organic challenge consisting of 0.3 % BSA (bovine serum albumin) is considered to be under clean conditions and the test with 3% BSA and 3% sheep erythrocytes is considered to be under dirty conditions.

[2] To record the efficacy limit values at least two concentrations (use concentration and an ineffective concentration) must be tested. (For gradations, see Chapter 5 of the VAH Methods and Requirements).

[3] Three of the listed contact times shall be tested, including the claimed contact times.

[4] Additionally for products with oxidative activity

[5] Test reports based on EN 14476:2013 continue to be valid.

Table V3.2: Test conditions in the simulated-use test.

Activity spectrum	Test organisms	Test method	Interfering substance[1]/ Test concentrations[2]	Test temperature [°C]	Contact times [min][3]
Active against enveloped viruses*	Vaccinia virus	EN 17111 [5]	Under clean or dirty conditions/ use concentration	20 ± 1	1, 5, 15, 30, 60
Virucidal instrument disinfection at < 40 °	Adenovirus Norovirus SV40	EN 17111 [5]	Under clean or dirty conditions/ use concentration	20 ± 1 to < 40 ± 1	
Virucidal instrument disinfection at ≥ 40 °C	Parvovirus	EN 17111 [5]	Under clean or dirty conditions/ see footnote[2]	≥ 40 ± 1 to ≤ 70 ± 1	

* For combined detergent/disinfectant precleaning products, suitable methods must be used to demonstrate the absence of protein-fixing properties (e.g. Amido black stain.

[1] The Interfering substance based on EN 17111 with 0.3% BSA (bovine serum albumin) is considered to be under clean conditions and the test with 3% BSA and 3% sheep erythrocytes is considered to be under dirty conditions.

[2] To record the efficacy limit at least two concentrations (use concentration and an ineffective concentration) must a be tested. (For gradations see Chapter 5).

[3] The test contact times are based on Table V3.3.

Table V3.3: The contact times to be selected in the individual test runs for simulated-use testing of instrument disinfectants.

Claimed contact time	1st test run	2nd test run
5 min	1 min, 5 min	5 min
15 min	5 min, 15 min	15 min
30 min	15 min, 30 min	30 min
60 min	30 min, 60 min	60 min

The simulated-use tests must be performed in all cases in two test runs:

1. Test run: In each case, at least two test surfaces per claimed concentration-time relation (see **Table V3.2** and **V3.3**), virus control (before and after drying), cytotoxicity test and reference test.
2. Test run: In each case, at least two test surfaces per claimed concentration-time relation, virus control (before and after drying), cytotoxicity test and reference test

In the second test run, only the most resistant test virus from the first test run needs to be tested for procedures < 40 °C.

Test viruses as per DVV/RKI guidelines and DIN EN 14476

Adenovirus = Adenovirus type 5, Adenoid 75 strain

BVDV = Bovine Viral Diarrhoea Virus, NADL strain

Norovirus = Murine norovirus, S99 Berlin (MNV) strain

Parvovirus = Murine parvovirus (minute virus of mice, rodent protoparvovirus 1) (MVM)

Poliovirus = Poliovirus vaccination type 1 strain, LSc-2ab strain

SV40 = Polyomavirus (SV 40), 777 strain

Vaccinia virus = Modified vaccinia virus Ankara (MVA) or vaccinia virus, Elstree strain

References

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V4A Chemothermal laundry disinfection

Requirements

For the conformity assessment procedure two independent expert opinions, including test reports based on DIN EN 14476 [1], confirming the efficacy in the claimed concentration-time relation in quantitative suspension tests must be submitted.

In addition, controls must be included to demonstrate the quality of the test viruses and the validity of the test. Since at present these controls are not adequately described in DIN EN 14476 or are missing in some cases, controls must be run as per DVV/RKI guideline 2015 [2] Subpara. 5.1 and 7.6 or DVV/RKI guideline 2008 [3] Subpara. 5.1 and 7.7.

The use concentration must be confirmed in the respective test report in a second independent test batch and, as controls, must include the virus control, cytotoxicity test and reference test. The mean confidence interval for two independent tests must each be ≤ 0.5 lg.

The bactericidal and yeasticidal activity confirmed by the VAH by means of a conformity assessment procedure or confirmed additionally during this procedure is a prerequisite for certification. The procedural sequence (e.g. time disinfectant added) should be taken into account in the virus tests as far as possible.

The concentration-time relation for activity against viruses featured in the VAH List must not be shorter than that specified for the VAH-listed bactericidal and yeasticidal efficacy. The test concentrations and contact times must be selected such that the relationship between the disinfectant virucidal activity and the concentration and contact time is evident from the test results (kinetics). It is recommended to test the concentration-time relations specified in **Table V4.1**. Other concentration-time relations – but at least three – are possible, provided that the kinetics and ineffective limit values are evident from these.

Obligatory:

- *Determination of the virucidal activity (virucidal activity in the quantitative suspension test as per DIN EN 14476 [1]).*

To that effect, the method must be tested with the complete procedure (i.e. all components* in one test batch) at 20 °C ± 1 °C and at the specified process temperature. The virus titres must also be determined at 20 °C ± 1°C and at the process temperature.

The test product must reduce the virus titre of the test viruses listed in **Table V4.1** under the specified conditions within the stipulated contact time(s) at the test temperature by at least 4 lg levels.

A reference and cytotoxicity test must be performed for each test run. If detoxification of the test batch is done with columns, the same test run must be carried out additionally in parallel without columns [see 2, Annex 7].

Table V4.1: Test conditions in the quantitative suspension test.

Activity spectrum	Test organisms	Test method	Interfering substance[1]/ Test concentrations[2]	Test temperature [°C]	Contact times [min]
Virucidal activity (chemo-thermal)	Parvorvirus[3]	DIN EN 14476, controls as per DVV/RKI [2, 3]	Under clean or dirty conditions/ see footnote[2]	≥ 30 ± 1 to ≤ 70 ± 1	5, 10, 15, 20

[1] Based on DIN EN 14476, the test batch with 3% BSA and 3% sheep erythrocytes is considered to be under dirty conditions.
Clean conditions – 0.3% BSA (bovine serum albumin) - may only be used for testing if the procedure includes a pre-wash step.

[2] To record the efficacy limit values at least two concentration-time relations (use concentration and an ineffective concentration) must be tested.

[3] Test reports with the bovine parvovirus continue to be valid if they meet the requirements set out here.

Table V4.2: Test conditions (test batch and controls).

Virus titre	Temperature		
	20 °C ± 1 °C	Process temperature	60 °C ± 1 °C
Virus control under clean conditions[1]	1 test run	2 test runs	
Virus control under dirty conditions	1 test run	2 test runs	
Test batch with all components	1 test run	2 test runs	
Reference substance * (peracetic acid 0.005%/ 10 min)			2 test runs

[1] Only required if the procedure includes a pre-wash step.

* Alternatively, testing as per DVV/RKI guideline 2008 [3], Subpara. 5.1 and 7.7 may also be accepted to demonstrate the quality of the test viruses (control with pH value of the wash process at 20 °C and at the process temperature).

* It is not necessary to test the individual components, e.g. detergent, laundry cleaning booster, disinfectant (in cases where the procedure concerned is a multi-component procedure)

Test viruses as per DVV/RKI guidelines and DIN EN 14476

Parvovirus = Murine parvovirus (minute virus of mice, rodent protoparvovirus 1) (MVM)

References

1. DIN EN 14476:2019-10. Chemische Desinfektionsmittel und Antiseptika – Quantitativer Suspensionsversuch zur Bestimmung der viruziden Wirkung im humanmedizinischen Bereich – Prüfverfahren und Anforderungen (Phase 2, Stufe 1); Deutsche und Englische Fassung EN 14476: 2013+A2: 2019. DIN Deutsches Institut für Normung e.V.:1–42.
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