

# TEST METHODOLOGY

## HYGIENIC HANDWASH

### Explanatory Notes on the Test Methodology

The following requirements must be met to certify hygienic handwash products [1, 2]:

Efficacy against bacteria and yeasts must be demonstrated.

1. To that effect, the following *orientational* tests must be conducted:

- Determination of bacteriostatic activity as well as of suitable neutralizers (Method 7).
- Determination of bactericidal efficacy in the qualitative suspension test (Method 8).

2. The following tests are *obligatory*:

- *Phase 2/Step 1*: Determination of bactericidal activity in the quantitative suspension test (Method 9) at 20 °C, with 50 % (final concentration in the test) and as a concentrate (in addition to the concentrations required for assessment of the activity limit) at 15 s, 30 s and 1 min.

The test product must reduce the number of the test organisms *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if this had proved to be more resistant than *P. aeruginosa* in the qualitative suspension test or if a qualitative suspension test is lacking) by at least 3 lg and *C. albicans* by at least 2 lg under dirty conditions within the recommended contact time (30 s or 1 min) at 20 °C.

- *Phase 2/Step 2*: Hygienic handwash – simulated-use test with volunteers (Method 10) (DIN EN 1499 [3]).

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

- Complete results suitable for evaluation must be available for at least 12 of the 15 volunteers and
- the logarithmic (lg) total mean value of the pre-values for the reference and test procedure(s) must be at least 5.

If the mean value of the lg reduction factor of the test procedure is significantly greater than that of the soap-based reference procedure, the test product meets the requirements under simulated-use conditions.

### Listing of Antiviral Properties

(For more detailed information, please also refer to the *General Preface*)

According to VAH Communications No. 3/2010 [4], the VAH Disinfectant Commission decided to conduct a conformity assessment procedure of antiviral effectiveness based on the test reports and test protocols and include antiviral properties in the VAH List of Disinfectants, if requested by the manufacturer. For hygienic handwash products the concentration-

contact time ratios for *activity against enveloped viruses* are listed in a separate row for the respective product, if applicable. Independent of the concentration/contact time ratios recommended in the test protocols for antiviral activity, the values for bactericidal and yeasticidal activity are always specified as minimum requirements for practical use. Therefore, antiviral activity values stated in the VAH List are never lower than the ones for bactericidal activity. For listing antiviral properties, manufacturers must provide two manufacturer-independent expert reports with test reports according to EN 14476 [5].

This information in the VAH List is given on an interim basis until a harmonised standard test and evaluation procedure for virucidal activity has been adopted in Europe which includes conditions simulating practical use.

### References

1. Desinfektionsmittel-Kommission im VAH (Eds.). Requirements and Methods for VAH Certification of Chemical Disinfection Procedures. Issue: April 2015. Wiesbaden: Mhp-Verlag, 2016 (Epub), updates in German or online [www.vah-online.de](http://www.vah-online.de).
2. Desinfektionsmittel-Kommission im VAH. Aktuelle Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. Stand 2. April 2015. Kommentar und Übergangsmodalitäten. HygMed 2015;40(6):268-269. (English translation available from VAH website: [www.vah-online.de](http://www.vah-online.de).)
3. DIN EN 1499:2017-10. Desinfizierende Händewaschung, Prüfverfahren und Anforderungen (Phase 2/Stufe 2). Berlin: Beuth Verlag GmbH 2017. English version: EN 1499:2013.
4. Desinfektionsmittel-Kommission im VAH: Listung viruswirksamer Eigenschaften in der VAH-Desinfektionsmittel-Liste. Mitteilung Nr. 3/2010. HygMed 2010; 35 (7/8):273.
5. 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); English Version: EN 14476:2013+A2:2019.

## HAND DISINFECTION

### Explanatory Notes on the Test Methodology

#### 1 Hygienic Hand Disinfection

The following minimum requirements must be met to certify products for hygienic hand disinfection [1, 2]:

Bactericidal (apart from mycobacteria) and yeasticidal (*C. albicans*) activity must be demonstrated.

1. To that effect, the following **orientational** preliminary tests must be conducted:

- Determination of bacteriostatic and yeaststatic activity as well as of suitable neutralizers (Method 7).
- Determination of bactericidal and yeasticidal activity in the qualitative suspension test (Method 8).

2. The following tests are **obligatory**:

- **Phase 2/Step 1:** Determination of bactericidal and yeasticidal activity (*C. albicans*) in the quantitative suspension test (Method 9) at 20 °C as a concentrate (in addition to the concentration required for assessment of the boundary region) using a high organic challenge at 15 s, 30 s and 1 min.

The test product must reduce the number of the test organisms *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if this has proved to be more resistant than *P. aeruginosa* in the qualitative suspension test or if no qualitative suspension test is carried out) under dirty conditions within the recommended contact time (30 s or 1 min) at 20 °C by at least 5 lg levels as well as of *C. albicans* by at least 4 lg levels. Thus, each product listed has demonstrated efficacy against bacteria and yeasts (*C. albicans*).

- **Phase 2/Step 2:** Hygienic hand disinfection – simulated-use test with volunteers (Method 11) (DIN EN 1500 [3]).

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

- Complete results must be available for at least 18 of the 20 volunteers and
- The total mean logarithmic value of the pre-values for the reference and test procedure(s) must be at least 5, and
- As a consequence of the stricter requirements on the statistical evaluation, the number of test results with a reduction <3 lg may be adjusted to the actual number of test subjects evaluated. For example, for 30 evaluated test subjects, an increase from 3 to 4 results with a reduction <3 lg is permitted.

The difference of the mean reduction values has to be tested for statistical significance. It has to be demonstrated that the test product is not significantly less effective than that of the propan-2-ol-based (60vol% application with 2x3 ml for 2x30s) reference procedure. The test is performed according to the Hodges&Lehmann procedure.

The Disinfectant Commission published a communication in 2020 [5] regarding hand disinfectants applied as foam. These foams, too, must be tested according to simulated-use conditions.

#### 2 Surgical Hand Disinfection

The following requirements must be met to certify products for surgical hand disinfection [1, 2]:

Bactericidal and yeasticidal (*C. albicans*) activity must be demonstrated.

1. To that effect, the following **orientational** preliminary tests must be conducted:

- Determination of bacteriostatic and yeaststatic activity as well as of suitable neutralizers (Method 7).
- Determination of bactericidal and yeasticidal activity in the qualitative suspension test (Method 8).

2. The following tests are **obligatory**:

- Determination of bactericidal efficacy in the quantitative suspension test (Method 9) at 20 °C, with 50 % (final concentration in the test) and as a concentrate (in addition to the concentration required for assessment of the boundary region) using a low organic challenge at a minimum contact time of 1 min, 3 min or 5 min. If the contact time recommended by the manufacturer differs from these test contact times, a separate, additional test has to be performed for this time.

The test product must reduce the number of test organisms of *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if these have proved to be more resistant than *P. aeruginosa* in the qualitative suspension test) under clean conditions within the recommended contact times (1–3 min, 5 min) at 20 °C by at least 5 lg as well as of *C. albicans* by at least 4 lg. **Thus, each product listed has demonstrated efficacy against bacteria and yeasts (*C. albicans*).**

- **Phase 2/Step 2:** Surgical hand disinfection – simulated-use test with volunteers (Method 12) (DIN EN 12791 [4]).

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

- Complete results must be available for at least 18 of the 20 volunteers and
- The total mean logarithmic value of the pre-values for the reference and test procedure(s) must be at least 3.5.

If the mean value of the lg reduction factor obtained for the immediate effect and the effect after 3 h of the test procedure is not significantly lower than that of the propan-1-ol-based reference procedure, the test product meets the requirements under simulated-use conditions.

The reference procedure is performed with 60 vol% propan-1-ol for 3 min. The test procedure may also be performed for shorter contact times, with a minimum of 1 min.

If, furthermore, the mean value of the lg reduction factor obtained for the sustained effect (after 3 h) of the test procedure is significantly greater than that of the propan-1-ol-based reference procedure, the test product meets the requirements under simulated-use conditions for a procedure endowed with “a sustained effect”.

## Listing of Antiviral Properties

(For more detailed information, please also refer to the General Preface)

According to the VAH Communication from August 2018 [6], the VAH Disinfectant Commission decided to conduct a conformity assessment procedure of antiviral effectiveness according to the test reports and test protocols and include antiviral properties in the VAH List of Disinfectants, if requested by the manufacturer. The concentration-contact time ratios for *activity against enveloped viruses*, *limited spectrum virucidal activity* and *virucidal activity* are listed in a separate row for the respective product, if applicable. Independent of the concentration-contact ratio recommended for activity against viruses in the experts reports, the values listed for bactericidal activity must be considered as the minimum requirement for practical use. Therefore, the antiviral activity values stated in the VAH List are never lower than the ones for bactericidal activity. Products with information on antiviral activity in the VAH List were subjected to a conformity assessment procedure corresponding to the current test methods [7–10] by independent experts of the Disinfectants Commission on the basis of at least one manufacturer-independent expert report and test protocol.

Testviruses according to the DVV/RKI Guideline and/or EN 14476 are:

- Adenovirus = Adenovirus Typ 5, strain Adenoid 75
- BVDV = Bovine Viral Diarrhea Virus, strain NADL
- Norovirus = Murines Norovirus, Stamm S99 Berlin (MNV)
- Parvovirus = Murines Parvovirus (Minute Virus of Mice) (MVM)
- Poliovirus = Poliovirus-Impfstamm Typ 1, strain LSc-2ab
- SV 40 = Polyomavirus (SV 40), strain 777
- Vacciniavirus = Modified Vacciniavirus Ankara (MVA) or Vacciniavirus, strain Elstree.

The following tests are *obligatory*: Determination of the antiviral activity (all activity spectrums) in the the quantitative suspension test (method DVV/RKI 2005, 2008 or 2015 [7, 8, 9]) or EN 14476 [10]. The test protocols have to comply with the requirements published by the Working Group „Antiviral Activity“ [11]. The product to be tested has to reduce the virus titre of the test organisms listed in **Table 1** under the prescribed conditions within the claimed contact time(s) at 20 °C by at least 4 lg.

This process will be in effect on an interim basis until a harmonised standard test and evaluation procedure for virucidal activity has been adopted in Europe which includes conditions simulating practical use.

## References

1. Desinfektionsmittel-Kommission im VAH (Eds.). Requirements and Methods for VAH Certification of Chemical Disinfection Procedures. Issue: April 2015. Wiesbaden: Mhp-Verlag, 2016 (Epub), updates in German.
2. Desinfektionsmittel-Kommission im VAH. Aktuelle Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. Stand 2. April 2015. Kommentar und Übergangsmodalitäten. HygMed 2015;40(6):268-269. (English translation available from VAH website).
3. DIN EN 1500:2017-10. Hygienische Händedesinfektion, Prüfverfahren und Anforderungen (Phase 2/Stufe 2). Berlin: Beuth Verlag GmbH, 2017. English version: EN 1500:2013.
4. DIN EN 12791:2018-01. Chirurgische Händedesinfektionsmittel, Prüfverfahren und Anforderungen (Phase 2/Stufe 2). Berlin: Beuth Verlag GmbH, 2018. English version: EN 12791:2016+A1:2017.
5. Desinfektionsmittel-Kommission im VAH (Hrsg.) Wirksamkeitsprüfung von alkoholischen Schäumen zur hygienischen Händedesinfektion. HygMed 2020;45(5):76–78.
6. Desinfektionsmittel-Kommission im VAH. Anforderung an die Zertifizierung von viruswirksamen Verfahren für die VAH-Liste - Modalitäten für die Antragstellung. HygMed 2018;43(7/8):141.

**Tabelle 1:** Test conditions for virucidal activity for hygienic hand disinfectants in quantitative suspension tests.

Activity Spectrum	Testorganisms	Test Method	Interfering substance <sup>1</sup> / Test concentrations <sup>2</sup>	Contact times <sup>3</sup> at 20 °C ± 1 °C
Active against enveloped viruses	Vacciniavirus BVDV <sup>4</sup>	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	DVV/RKI interfering substance or clean conditions (EN)/ undiluted	15 s, 30 s, 1 min
Limited spectrum virucidal activity	Adenovirus Norovirus	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	DVV/RKI interfering substance or clean conditions (EN)/ undiluted	15 s, 30 s, 1 min 1.5 min, 2 min
Active against enveloped and non-enveloped viruses (virucidal)	Poliovirus Adenovirus Norovirus SV 40	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	DVV/RKI interfering substance or clean conditions (EN)/ undiluted	15 s, 30 s, 1 min, 1.5 min, 2 min

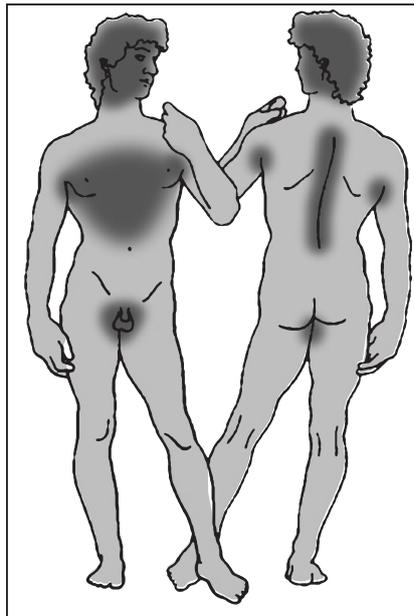
<sup>1</sup> The interference substance according to DVV/RKI is 10 % FCS (fetal calf serum) and A dest., respectively, or, according to DIN EN 14476 0.3 % BSA (bovine serum albumin) for clean conditions.

<sup>2</sup> in addition to the required dilutions for recording the efficacy limits (at least 3 concentrations)

<sup>3</sup> At least three of the contact times have to be tested, including the claimed contact times. The contact times are specified in the test methods and should be between 15 s and 2 min. The contact time should be ≤ 60 s at most.

<sup>4</sup> Additionally for products with oxidative activity

7. DVV, RKI. Leitlinie der DVV und des RKI zur Prüfung von chemischen Desinfektionsmitteln gegen Viren in der Humanmedizin. Bundesgesundheitsbl 2005;48:1420–1426.
8. DVV, RKI. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Instituts (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (Fassung vom 1. 8. 2008). HygMed 2008;33:315–322.
9. DVV, RKI. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Instituts (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin – Fassung vom 1. Dezember 2014. Bundesgesundheitsbl 2015;58:493–504.
10. 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); Berlin: Beuth Verlag GmbH, 2019 .English version EN 14476:2013+A2:2019.
11. Schwebke I, Eggers M, Gebel J, Geisel B, Glebe D, Rapp I, Steinmann J, Rabenau HF. Prüfung und Deklaration der Wirksamkeit von Desinfektionsmitteln gegen Viren zur Anwendung im human-medizinischen Bereich. Stellungnahme des Arbeitskreises Viruzidie beim Robert Koch-Institut. Bundesgesundheitsbl 2017; 60:353–363.



Skin with a low density of sebaceous glands (Ø100 sebaceous glands/cm<sup>2</sup>)  
 Skin with a high density of sebaceous glands (400 bis 900 sebaceous glands/cm<sup>2</sup>)

**Figure 1:** Density of sebaceous glands on human skin. For the palms of the hands, the recommendations for hand disinfection apply.

## SKIN ANTISEPSIS

### Explanatory Notes on the Test Methodology

The following requirements must be met to certify products for skin antiseptics [1, 2]:

Bactericidal and yeasticidal (*C. albicans*) activity must be demonstrated.

1. To that effect, the following **orientational** preliminary tests must be conducted:

- Determination of bacteriostatic and yeaststatic activity as well as of suitable neutralizers (Method 7).
- Determination of bactericidal and yeasticidal activity in the qualitative suspension test (Method 8).

2. The following tests are **obligatory**:

- **Phase 2/Step 1:** Determination of bactericidal and yeasticidal activity (*C. albicans*) in the quantitative suspension test (Method 9) at 20 °C under dirty conditions at 15 s, 30 s, 1 min, 3 min, 5 min or 10 min.

The test product must reduce the number of the test organisms *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if this proved to be more resistant than *P. aeruginosa* in the qualitative suspension test or if no qualitative suspension test is carried out) within the recommended contact times (15 s, 30s, 1 min, 3 min, 5 min and 1 min, 1.5 min, 2 min, 2.5 min, 3 min, 5 min, 10 min) at 20 °C by at least 5 lg levels as well as of *Candida albicans* by at least 4 lg levels [1]. **Thus, each product listed has demonstrated efficacy against bacteria and yeasts (*C. albicans*).**

The following test method is **obligatory** for evaluating the efficacy against the **resident skin flora** [1]:

- **Phase 2/Step 2:** Skin antiseptics (Method 13)

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

- Complete results must be available for at least 18 of the 20 volunteers.
- The total mean logarithmic values of the pre-values has to be > 2.

If the mean value(s) of the reduction factor of the test procedure at the pertinent contact times is/are smaller than those of the reference procedure and if the difference is statistically corroborated for at least one mean value, the test product shall be rejected as being unsuitable.

This applies for skin that has both a low and a high density of sebaceous glands: For declaration of efficacy on skin that has a low density of sebaceous glands, tests are carried out on the upper arms of volunteers, and on the forehead for declaration of efficacy on skin that has a high density of sebaceous glands (cf. **Figure 1**). The skin is not artificially contaminated with test bacteria for these tests; rather, proof of efficacy is furnished on the basis of the existing bacterial contamination levels of the skin of each volunteer.

### References

1. Desinfektionsmittel-Kommission im VAH (Eds.). Requirements and Methods for VAH Certification of Chemical Disinfection Procedures. Issue: April 2015. Wiesbaden: Mhp-Verlag, 2016 (Epub), updates in German (June 2019).
2. Desinfektionsmittel-Kommission im VAH. Aktuelle Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. Stand 2. April 2015. Kommentar und Übergangsmodalitäten. HygMed 2015;40(6):268-269. (English translation available from VAH website)

## SURFACE DISINFECTION

### Introductory Remarks

The application field “Surface” exclusively lists bactericidal and yeasticidal (*C. albicans*) products tested as per the “Requirements and Methods for VAH Certification of Chemical Disinfection Procedures” [1] or the transitional provisions [2].

In accordance with European standards, tests for surface disinfectants are conducted with a low and a high organic challenges in quantitative suspension tests and in tests reflecting everyday use conditions (contaminated germ carriers). Furthermore, a distinction is made between applications that involved “no mechanical action” and “with mechanical action” (scrub-wipe disinfection). Efficacy against *S. aureus*, *E. hirae*, *P. aeruginosa*, *E. coli*\*, *P. mirabilis*\* (to be tested in the quantitative suspension test, if seen to be more resistant than *P. aeruginosa* in the qualitative suspension test or if no qualitative test is carried out) and *C. albicans* must be proven for each concentration/contact time ratio specified for a product in this section.

Efficacy against *A. brasiliensis*, *M. terrae* and *M. avium* can be claimed as an additional option.

### Explanatory Notes on the Test Methodology

#### Bacteria and Fungi

1. The following preliminary tests must be conducted as an *orientational* guide for certification of products for surface disinfection:

- Determination of bacteriostatic and yeastic activity as well as of suitable neutralizer (Method 7) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).
- Determination of bactericidal and yeasticidal (*C. albicans*) activity in the qualitative suspension test (Method 8) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).

2. The following tests are *obligatory*:

- *Phase 2/Step 1*: Determination of bactericidal and yeasticidal (*C. albicans*) activity in the quantitative suspension test (Method 9) (test conditions see **Table 2**).

The test product must reduce the number of the test organisms *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if this proved to be more resistant than *P. aeruginosa* in the qualita-

tive suspension test or if no qualitative test is carried out) under clean and/or dirty conditions within the specified contact times (5, 15, 30, 60 or 240 min) at 20 °C by at least 5 lg as well as the number of *C. albicans* by at least 4 lg. The test organisms *M. terrae*, *M. avium*, *A. brasiliensis*, which can be tested as an optional, additional claim, must be reduced by at least 4 lg.

- *Phase 2/Step 2*: Investigation of bactericidal and yeasticidal efficacy under practical conditions (Method 14.1 without mechanical action and Method 14.2 with mechanical action [1, 2, 3, 4]).

> Two series of tests must be run in each case.

> Consult **Table 2** for the requirements addressed to this test procedure. The test product has to reduce the test organisms *S. aureus*, *E. hirae* and *P. aeruginosa* under low or high organic load within the specified contact times (5, 15, 30, 60 or 240 min) at 20 °C by at least 5 lg or for *C. albicans* by at least 4 lg.

*Thus, each product listed has demonstrated efficacy against bacteria and yeasts (C. albicans).*

The test organisms *M. terrae*, *M. avium*, *A. brasiliensis*, which can be tested as an optional, additional claim, must be reduced by at least 4 lg.

#### Efficacy against Fungi on Untreated Wood

*Optional tests are:*

- Germ carrier test on untreated wood for determination of the fungicidal efficacy (Method P1 [1, 2]).

When using the recommended concentration/contact-time ratio, there must not be evidence of fungal growth during the test.

### Listing of Antiviral Properties

*(For more detailed information, please also refer to the General Preface)*

As stated in the VAH Communication No. 3/2010 [5], the VAH Disinfectant Commission decided to conduct a conformity assessment procedure to confirm the antiviral effectiveness of

Area	Organic Load/ Contamination	Contact Times	Required Reduction
Surface disinfection with and/or without mechanical action	<i>Clean conditions</i> 0.03 % albumin	1 min 5 min 15 min 30 min 60 min 240 min	5 lg for bacteria (Exception: 4 lg for <i>P. aeruginosa</i> 60 or 240 min <sup>1</sup> ) 4 lg for <i>C. albicans</i> (Exception: 3 lg for <i>C. albicans</i> 60 or 240 min <sup>1</sup> )
	<i>Dirty conditions</i> 0.3 % albumin and 0.3 % sheep erythrocytes		<i>Optional:</i> 4 lg for mycobacteria 4 lg for <i>A. brasiliensis</i> 4 lg for viruses

**Table 2:** Test conditions and required lg reduction for surface disinfectants in the quantitative suspension test and under simulated-use conditions.

<sup>1</sup> This reduction is permissible in the test conducted under practical conditions if the control values have dropped because of test organisms dying to a level where a 5 lg reduction cannot be demonstrated anymore.

products, if this was requested by the manufacturer (cf. General Preface). Details of the certification process are specified in the Standard Methods and Requirements (2nd amendment of 25 April 2018) [1, 6].

The concentration-contact time ratios for *activity against enveloped viruses, limited spectrum virucidal activity* and *virucidal activity* are listed in a separate row for the respective product, if applicable. Independent of the concentration-contact ratio recommended for activity against viruses in the experts reports, the values listed for bactericidal activity must be considered as the minimum requirement for practical use. Therefore, the antiviral activity values stated in the VAH List are never lower than the ones for bactericidal activity. Products with information on antiviral activity in the VAH List were subjected to a conformity assessment procedure corresponding to the current test methods [7–12] by independent experts of the Disinfectants

Commission on the basis of at least one manufacturer-independent expert report and test protocol. The test protocols have to comply with the requirements published by the Working Group „Antiviral Activity“ [13].

*The following tests are obligatory:* Determination of the antiviral activity (all activity spectrums) in the quantitative suspension test [7–10] and in tests simulating use-conditions [11, 12].

The simulated-use tests shall be performed in all cases in two independent test runs with in each case 3 test surfaces each per contact-time-ratio and per WSH control (Tables 3 and 4). In the second test run 3 test surfaces per claimed concentration-time relation and per WSH control shall be included.

Testviruses according to the DVV/RKI Guidelines and/or EN 14476 [7–10 and 11, 12, respectively] are:

– Adenovirus = Adenovirus Typ 5, strain Adenoid 75

**Table 3:** Test conditions for virucidal activity for surface disinfectants in quantitative suspension tests.

Activity Spectrum	Testorganisms	Test Method	Interfering substance <sup>1</sup> / Test concentrations <sup>2</sup>	Test temperature [°C]	Contact times <sup>3</sup> [min]
Active against enveloped viruses	Vacciniavirus BVDV <sup>4</sup>	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1	1, 5, 15, 30, 60, 240
Limited spectrum virucidal activity	Adenovirus Norovirus	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1	
Active against enveloped and non-enveloped viruses (virucidal)	Poliovirus Adenovirus Norovirus SV 40	DVV/RKI [7, 8, 9] or DIN EN 14476 [10]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 to < 40 °C	

<sup>1</sup> The interfering substance based on DVV/RKI is 10 % FCS (fetal calf serum) and distilled water or, based on DIN EN 14476, clean conditions mean an interfering substance of 0.3 % BSA (bovine serum albumin). The test with 3% BSA and 3% sheep erythrocytes is considered to be a test under dirty conditions.

<sup>2</sup> At least 3 different concentrations must be tested, with one of the concentration being in the effective range and one concentration in the ineffective range.

<sup>3</sup> The contact times are specified in the test methods and should not be longer than 240 min. At least two contact times must be tested.

<sup>4</sup> Additionally for products with oxidative activity

**Table 4:** Test conditions for virucidal activity for surface disinfectants in tests simulating use-conditions.

Activity Spectrum	Testorganisms	Test Method	Interfering substance <sup>1</sup> / Test concentrations <sup>2</sup>	Test temperature [°C]	Contact times <sup>3</sup> [min]
Active against enveloped viruses	Vacciniavirus BVDV <sup>4</sup>	DVV Guideline 2012 [11] or EN 16777 [12]	Clean or dirty conditions/ see footnote <sup>2</sup>	22 ± 3	1, 5, 15, 30, 60, 240
Limited spectrum virucidal activity (low level)	Adenovirus Norovirus	DVV Guideline 2012 [11] or EN 16777 [12]	Clean or dirty conditions/ see footnote <sup>2</sup>	22 ± 3	
Active against enveloped and non-enveloped viruses (high level)	Adenovirus* Norovirus* Parvovirus	DVV Guideline 2012 [11] or EN 16777 [12]	Clean or dirty conditions/ see footnote <sup>2</sup>	22 ± 3	

<sup>1</sup> The test with 0.3% BSA (bovine serum albumin) is considered to be performed under clean conditions, the test with 3% BSA and 3% sheep erythrocytes under dirty conditions.

<sup>2</sup> At least 3 different concentrations must be tested, with one of the concentration being in the effective range and one concentration in the ineffective range.

<sup>3</sup> The contact times are specified in the test methods and should not be longer than 240 min. Apart from the contact time to be certified the next shorter contact times has to be tested in the first test run. In the second test run the claimed concentration-contact time is confirmed with 2 test surfaces.

<sup>4</sup> Additionally for products with oxidative activity

\* If effectiveness for the claim low level virucidal activity has not been proven previously

- BVDV = Bovine Viral Diarrhea Virus, strain NADL
- Norovirus = Murines Norovirus, Stamm S99 Berlin (MNV)
- Parvovirus = Murines Parvovirus (Minute Virus of Mice) (MVM)
- Poliovirus = Poliovirus-Impfstamm Typ 1, strain LSc-2ab
- SV 40 = Polyomavirus (SV 40), strain 777
- Vacciniavirus = Modified Vacciniavirus Ankara (MVA) or Vacciniavirus, strain Elstree.

## References

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## INSTRUMENT DISINFECTION

### Introductory Remarks

Instrument disinfectants presently denote disinfectants used for immersion (manual disinfection). The VAH list only contains instrument disinfectants which are bactericidal and yeasticidal (*C. albicans*) tested as per tested as per the “Requirements and Methods for VAH Certification of Chemical Disinfection Procedures” [1] or the transitional provisions [2]. Details are laid down in the amendment to these methods and requirement published as of 15 June 2019.

The methodology in the “Requirements and Methods” [1] is in accordance with the European Standards. For efficacy testing, the results of quantitative suspension tests and of tests conducted under every-day use conditions (contaminated frosted glass germ carriers as a model for medical devices) with low and/or high organic challenge have to be presented for the test organisms against which the product is claimed to be active.

### Explanatory Notes on the Test Methodology

#### Bacteria and Fungi

1. The following preliminary tests must be conducted as an *orientational guide* for certification of products for instrument disinfection:

- Determination of bacteriostatic and yeastistatic activity as well as of suitable neutralising agents (Method 7) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).
- Determination of bactericidal and yeasticidal activity in the qualitative suspension test (Method 8) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).

2. The following tests are *obligatory*:

- *Phase 2/Step 1*: Determination of bactericidal and yeasticidal activity in the quantitative suspension test (Method 9).

Under clean and/or dirty conditions, the test product must reduce the number of the test bacteria of *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\*, *E. coli*\* (\*if this proved to be more resistant in the qualitative suspensions test than *P. aeruginosa* or if no qualitative suspension test is carried out) within the specified contact times (5, 15, 30 or 60 min) at 20 °C by at least 5 lg levels as well as of *C. albicans* by at least 4 lg levels. The test organisms *M. terrae*, *M. avium*, *A. brasiliensis*, which can be tested as an optional, additional claim, must be reduced by at least 4 lg.

- Phase 2/Step 2: Determination of bactericidal and yeasticidal activity under everyday use conditions (Method 15).
  - > Two series of tests must be run in each case.
  - > Please consult **Table 5** for the requirements addressed to this test procedure.
- Thus, each product listed has demonstrated efficacy against bacteria and yeasts (*C. albicans*).

### Listing of Antiviral Properties

(For more detailed information, please refer to the General Preface)

According to the VAH Communication No. 3/2010 [3], the VAH Disinfectant Commission decided to conduct a conformity assessment of antiviral effectiveness according to the test reports and test protocols and include antiviral properties in

Area	Organic Load/Contamination	Contact Times	Required R
Instrument disinfection	Clean conditions 0.03% albumin	5 min 15 min 30 min 60 min	5 lg for bacteria
	and/or  Dirty conditions 0.3% albumin and 0.3% sheep erythrocytes		4 lg for <i>C. albicans</i>  Optional: 4 lg for mycobacteria 4 lg for <i>A. brasiliensis</i> 4 lg for viruses

**Table 5:** Test conditions and required reduction (R) for instrument disinfectants in the quantitative suspension test and under simulated-use conditions.

**Table 6:** Test conditions for virucidal activity for instrument disinfectants in quantitative suspension tests.

Activity Spectrum	Testorganisms	Test Method	Interfering substance <sup>1</sup> / Test concentrations <sup>2</sup>	Test temperature [°C]	Contact times <sup>3</sup> [min]
Active against enveloped viruses*	Vacciniavirus BVDV <sup>4</sup>	DVV/RKI [4, 5 bzw. 6] oder DIN EN 14476 [7]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1	1, 5, 15, 30, 60
Active against enveloped and non-enveloped viruses (virucidal) < 40 °C	Poliovirus Adenovirus Norovirus SV 40	DVV/RKI [4, 5 bzw. 6] oder DIN EN 14476 [7]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1 up to < 40 ± 1	
Active against enveloped and non-enveloped viruses (virucidal) ≥ 40 °C	Parvovirus	DVV/RKI [4, 5 bzw. 6] oder DIN EN 14476 [7]	Clean or dirty conditions/ see footnote <sup>2</sup>	≥ 40 ± 1 up to ≤ 70 ± 1	

\* Tests for products intended for precleaning with a combined cleaners/disinfectant must include proof that no protein-fixing properties are exhibited, using a suitable method (such as amido black staining).

<sup>1</sup> The interfering substance based on DVV/RKI is 10 % FCS (fetal calf serum) and distilled water or, based on DIN EN 14476, clean conditions mean an interfering substance of 0.3 % BSA (bovine serum albumin). The test with 3% BSA and 3% sheep erythrocytes is considered to be a test under dirty conditions.

<sup>2</sup> At least 3 different concentrations must be tested, with one of the concentration being in the effective range and one concentration in the ineffective range.

<sup>3</sup> The contact times are specified in the test methods and should not be longer than 60 min. At least two contact times must be tested.

<sup>4</sup> Additionally for products with oxidative activity.

**Table 7:** Test conditions for virucidal activity for instrument disinfectants in tests simulating use-conditions.

Activity Spectrum	Testorganisms	Test Method	Interfering substance <sup>1</sup> / Test concentrations <sup>2</sup>	Test temperature [°C]	Contact times <sup>3</sup> [min]
Active against enveloped viruses	Vacciniavirus BVDV <sup>4</sup>	EN 17111 [8]]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1	1, 5, 15, 30, 60
Active against enveloped and non-enveloped viruses (high level) < 40 °C	Adenovirus Norovirus SV 40	EN 17111 [8]	Clean or dirty conditions/ see footnote <sup>2</sup>	20 ± 1 up to < 40 ± 1	
Active against enveloped and non-enveloped viruses (high level) ≥ 40 °C	Parovirus	EN 17111 [8]	Clean or dirty conditions/ see footnote <sup>2</sup>	≥ 40 ± 1 up to ≤ 70 ± 1	

\* Tests for products intended for precleaning with a combined cleaners/disinfectant must include proof that no protein-fixing properties are exhibited, using a suitable method (such as amido black staining).

<sup>1</sup> The interfering substance in accordance with EN 17111 is 0.3 % BSA (bovine serum albumin) for clean conditions. The test with 3% BSA and 3% sheep erythrocytes is considered to be a test under dirty conditions.

<sup>2</sup> At least 3 different concentrations must be tested, with one of the concentration being in the effective range and one concentration in the ineffective range.

<sup>3</sup> The contact times are specified in the test methods and should not be longer than 60 min. Apart from the contact time to be certified the next shorter contact times has to be tested in the first test run. In the second test run the claimed concentration-contact time is confirmed with 2 test surfaces.

<sup>4</sup> Additionally for products with oxidative activity.

the VAH List of Disinfectants, if requested by the manufacturer. Details of the certification process are specified in the Standard Methods and Requirements (amendment of 15 June 2019) [1].

The concentration-contact time ratios for *activity against enveloped viruses* and *activity against enveloped and non-enveloped viruses (high level)* are listed in a separate row for the respective product, if applicable. Independent of the concentration-contact ratio recommended for activity against viruses in the experts reports, the values listed for bactericidal activity must be considered as the minimum requirement for practical use. Therefore, the antiviral activity values stated in the VAH List are never lower than the ones for bactericidal activity.

Products with information with respect to their antiviral activity in the VAH List were subjected to a conformity assessment procedure corresponding to the current test methods [4–8] by independent experts of the Disinfectants Commission on the basis of at least one manufacturer-independent expert report and test protocol.

*The following tests are obligatory:* Determination of the antiviral activity (against enveloped viruses and against enveloped as well as non-enveloped viruses (high level)) in the quantitative suspension test (Methods according to guidelines DVV/RKI 2005, 2008 or 2015 [4, 5, 6]) or DIN EN 14476 [7]. Determination of the antiviral activity in the carrier test simulating use-conditions according to DIN EN 17111 [8].

The product to be tested must reduce the titre of the test organisms listed in *Tables 6 and 7* under the prescribed conditions within the requisite contact time(s) and temperature(s) by at least 4 lg.

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

1st Test run: 2 test surfaces per concentration-contact time ration and 2 test surfaces per WSH control,

2nd Test run: 2 test surfaces per concentration-contact time ration and 2 test surfaces per WSH control,

In the second test run for processes  $\geq 40$  °C only the most resistant test virus from the first test run has to be tested.

Test viruses according to DVV/RKI-guidelines or DIN EN 14476:

- Adenovirus = Adenovirus Typ 5, strain Adenoid 75
- BVDV = Bovine Viral Diarrhea Virus, strain NADL
- Norovirus = Murines Norovirus, Stamm S99 Berlin (MNV)
- Parvovirus = Murines Parvovirus (Minute Virus of Mice) (MVM)
- Poliovirus = Poliovirus-Impfstamm Typ 1, strain LSc-2ab
- SV 40 = Polyomavirus (SV 40), strain 777
- Vacciniavirus = Modified Vacciniavirus Ankara (MVA) or Vacciniavirus, strain Elstree.

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## LINEN (TEXTILE) DISINFECTION

### Introductory Remarks

This List of certified products contains chemical-thermal single-chamber processes. In addition, chemical textile disinfectants for immersion may be listed according to item 1 “Chemical Linen Disinfection” in the explanatory notes on the test methods.

All disinfection procedures  $\geq 60$  °C are bactericidal, yeasticidal and tuberculocidal and were tested in quantitative suspension tests as well as in tests conducted under simulated-use conditions.

### Explanatory Notes on the Test Methodology

#### 1. Chemical Linen Disinfection

##### Immersion Procedures (Method 16)

A procedure has been deemed effective if in the working dilution at 13 °C the test organisms were inactivated on the test carriers within a maximum of 4–12 h [1–3].

#### 2. Chemical-thermal Linen Disinfection

(with mechanical action in a washing machine (Method 17) [1–3])

### Bacteria and Fungi

1. For certification of processes at temperatures 30° to < 60 °C, the following preliminary tests must be carried out as an **orientational** guide:

- Determination of bacteriostatic and yeaststatic (*C. albicans*) activity as well as of suitable neutralising agents (Method 7) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).
- Determination of bactericidal and yeasticidal (*C. albicans*) activity in the qualitative suspension test (Method 8) (not for *M. terrae*, *M. avium*, *A. brasiliensis*).

2. The following tests are **obligatory** for processes 30° to < 60 °C:

- Phase 2/Step 1: Determination of bactericidal and yeasticidal (*C. albicans*) activity in the quantitative suspension test (Method 9).

Using a low (procedures **with prewash**) or high organic challenge (procedures **without prewash**), the test product must reduce the numbers of the test organisms *S. aureus*, *E. hirae*, *P. aeruginosa*, *P. mirabilis*\* and *E. coli*\* (\*if these had proved to be more resistant than *P. aeruginosa* or if a qualitative suspension test is lacking) within the specified contact times (10, 15 or 20 min) at the process temperature by at least 5 lg as well as of *C. albicans* by at least 4 lg.

The microorganisms *M. terrae* and *M. avium* which can be tested as an optional efficacy claim must be reduced by at least 4 lg.

- Phase 2/ Step 2: Testing textile disinfection procedures at temperatures 30 °C to < 60 °C (Method 17.1) [1, 3].

A chemical-thermal textile disinfection procedure (single-chamber process) is deemed effective if it reduces the test organisms (*S. aureus*, *E. hirae*, *E. coli*, and *P. aeruginosa*) on the test receptacles by more than 7 lg (*C. albicans*, 6 lg, *A. brasiliensis* (optional), 6 lg, and *M. terrae* and/or *M. avium* (optional), by 7 lg) by using the recommended dosage, contact time and temperature as well as the specified liquor ratio. Nor should any microorganisms be detected in 100 mL washing solution after completion of the disinfection phase.

**Thus, each product listed for procedures between 30 °C and < 60 °C has demonstrated efficacy against bacteria and yeasts (*C. albicans*).**

3. The following tests are **obligatory** for processes ≥ 60 to 70 °C:

- Phase 2/Step 1: Determination of bactericidal and yeasticidal (*C. albicans*) activity in the quantitative suspension test at indicated process temperature (Method 9).

Using a low (procedures **with prewash**) or high organic challenge (procedures **without prewash**), the test product must reduce the colony count of the test bacterium *E. faecium* within the specified contact times (10, 15 or 20 min) at the indicated process temperature by at least 5 lg.

- Phase 2/Step 2: Testing linen disinfection procedures at temperatures ≥ 60 °C to 70 °C (Method 17.2) [1,3].

A chemical-thermal linen disinfection procedure (single-chamber process) is deemed effective if it reduces *E. faecium* in the test receptacles by more than 7 lg within the specified time by using the recommended dosage, contact time and

temperature as well as the specified liquor ratio.

Nor should any microorganisms be detected in 100 mL washing solution after completion of disinfection phase.

**As a result of the chemical and thermal resistance of *E. faecium* each product listed for disinfection procedures between ≥ 60 °C to 70 °C has demonstrated efficacy against bacteria and yeasts (*C. albicans*), mycobacteria, *M. tuberculosis* and fungi.**

### Listing of Antiviral Properties

*(For more detailed information, please refer to the General Preface)*

According to the VAH Communication No. 3/2010 [4], the VAH Disinfectant Commission decided to conduct a conformity assessment of antiviral effectiveness according to the test reports and test protocols and include antiviral properties in the VAH List of Disinfectants. For textile disinfectants **virucidal** activity may be claimed.

The concentration-contact time ratios for **virucidal** activity are listed in a separate row for the respective product. Independent of the concentration-contact ratio recommended for activity against viruses in the experts reports, the values listed for bactericidal activity must be considered as the minimum requirement for practical use. Therefore, the antiviral activity values stated in the VAH List are never lower than the ones for bactericidal activity. Products with information on antiviral activity in the VAH List were subjected to a conformity assessment procedure by independent experts of the Disinfectants Commission on the basis of at least one manufacturer-independent expert report and test protocol [5–7].

This process will be in effect on an interim basis until a harmonised standard test and evaluation procedure for virucidal activity has been adopted in Europe which includes conditions simulating practical use.

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*For further information, please visit the VAH website*  
<https://vah-online.de/en/expertise>

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