December 10, 2018, epub ahead of print (© mhp Verlag)

Surface disinfection against *Clostridium difficile* spores

19

19 Surface disinfection with mechanical action – simulate-use test (4-field-test)

(Method 19)

Testing the sporicidal activity on non-porous surfaces with mechanical action

19a Evaluation of the effectiveness of a disinfection solution applied by wiping with a standardised cloth material

In this test the main focus is placed on the evaluation of the **effectiveness of the disinfectant** with regard to the successful disinfection of surfaces, including floors, by wiping with a **standardised cloth material**.

Products applied by the user with any, unspecified cloth material – either diluted (from a concentrate to be diluted) or as a (ready-to-use RTU) solution) – must be tested according to 19a.

19b Evaluation of the effectiveness of the combination of a specified cloth material and a disinfectant

In this test the main focus is placed on the evaluation of the **effectiveness of a combination of the disinfectant with a specified cloth material** with regard to the successful disinfection of surfaces, including floors.

Products used as an impregnated wipe system – in which a dry cloth material specified by the product manufacturer is impregnated by the user with the product solution – must be tested according to 19b.

Products supplied to the user by the product manufacturer as ready-to-use wipe systems must also be tested according to 19b. For this method tested according to 19b, the second test run is carried out at the end of the claimed reuse period.

When testing according to 19b, the following additional information must be recorded in the test report:

A1 Wipe composition (if applicable, coating)

A2 Wipe size

A3 Grammage g/cm³

B Impregnation volume per wipe

C Use period if this is to be claimed for more than one working day.

December 10, 2018, epub ahead of print (© mhp Verlag)

19.1 Test organism and initial (baseline) concentrations

Clostridium difficile R027	NCTC 13366 (DSM 27147)	1.5 – 5 x 10^7 cfu/ml

Details on the preparation of stock and working cultures as well as the test suspensions are given in ➡ Chapter **18**. Before using the test suspension, the purity of the test suspension must be checked and documented using phase contrast microscopy.

The number of viable spores in the test suspension is adjusted to 1.5 – 5.0 x 10^7 per ml using a suitable method (➡ *Method 18.1.5*).

To simulate practical conditions, 0.03 % albumin (➡ *Appendix A1.8* – clean conditions) or 0.3 % Albumin and 0.3 % sheep erythrocytes (➡ *Appendix A1.8* – dirty conditions) is added to the test suspensions at the latest 2 h prior to the test.

19.2 Product test solution

Details on the preparation of the product test solution are given in ➡ Chapter **5**.

19.3 Test times

Select 1, 5, 15, 30, 60 and/or 240 min as test times.

19.4 Materialien

19.4.1 Test surfaces

PVC flooring with the dimensions 20 x 50 cm, 2 mm thick (e.g. FOREX classic, white, one foil-frosted surface, Mat. No.: SFSFOXC020RWH1F, thyssen-krupp Plastics GmbH, Widdersdorfer Strasse. 158, 50825 Cologne) is suitable as the test surface. Preclean the foil-free surface with 70% n-propanol without additional additives.

Four test fields are marked on the test surface as squares measuring 5 x 5 cm in a row 5 cm apart from each another and labelled 1 – 4 with a pencil or permanent marker. The row should be situated in the centre of the test surface and the first field should be 10 cm off the edge of the test surface (➡ *Figure 19.1*).

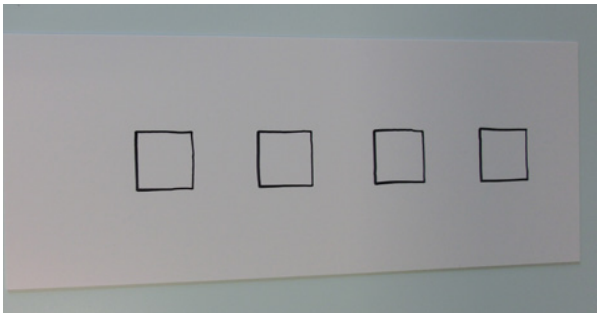

Figure 19.1: PVC-free foam board (FOREX classic).

To assure uniform precleaning a standard wiping cloth is impregnated with 16 ml 70% n-propanol (v/v) and, using the unitary weight, is wiped once back and forth over the test surface.

Once dry, the four test fields are marked on the test surface as squares measuring 5 x 5 cm in a row 5 cm apart from each another and labelled (T1–T4) with a pencil or permanent marker. The row should be situated in the centre of the test surface and the first field should be 10 cm off the edge (➡ *Figure 19.3*).

All test surfaces are kept in a horizontal position throughout the entire test. The relative humidity and the ambient air temperature (20 °C ± 1 °C) measured in the test room should be stated in the report. Two additional test fields are marked on smaller test surfaces (minimum 7 cm x 13 cm) as squares measuring 5 x 5 cm for the drying controls T0 and Tt.

19.4.2 Materials for the wiping procedure

Standard wiping cloth (wipe): For testing according to 19a and for the WSH control (➡ *19.10.2*) a standard wiping cloth with the dimensions 16.5 cm x 30 cm and composed of 55% pulp, 45% polyethylene terephthalate (PET) is used (e.g. Tork Low-Lint Cleaning Cloth, Art. No. 190491, SCA Tork). The wipes are used only once.

Specified wiping cloth (wipe): For impregnated wipe systems or ready-to-use wipe systems (➡ *19b*) the specified wiping cloth is used in combination with the disinfectant. The manufacturer is responsible for providing clear instructions for using the wipes (e.g. number of ply). The wipes are used only once.

Petri dishes: The standard wiping cloths are pre-moistened with 16 ml of the product test solution or WSH in a petri dish.

Unitary weight: Granite block (2.3 – 2.5 kg) with the dimensions 12.1 cm x 8.6 cm x 8.6 cm length x width x height). The height can vary depending on the thickness of the material. Use of the unitary weight standardises the wiping procedure and simulates the average contact pressure when wiping is performed in practice.

Rubber band: For securing the wipe on the unitary weight/block (e.g. Alco rubber band).

Parafilm (for single use only): Parafilm is used to protect the lower horizontal and vertical surfaces of the unitary weight from any form of contamination during the wiping procedure. The Parafilm must be renewed after each wiping procedure (e.g. Parafilm® M (100 mm) Art. No. 7016 05, BRAND GMBH + CO KG, Postfach 11 55, D-97861 Wertheim).

Spatula made of metal, glass or plastic with an edge length of 3 cm for distributing the test suspension when contaminating the test surfaces.

Swab (sterile, single-use): with a soakable component made of pure cotton and free of substances which might inhibit or promote the action of the test product or inactivate the test organisms.

December 10, 2018, epub ahead of print (© mhp Verlag)

19.5 Contamination of test field 1

A test surface is prepared for every product solution, contact time and interfering substance to be tested (➡ *19.4.1*).

0.1 ml of the interfering substance is mixed with 0.9 ml of the test suspension. 0.05 ml of this mixture (inoculum) is applied to test field 1 with a pipette and, using a spatula, is distributed evenly over the entire test field measuring 5 x 5 cm. The test surface is kept at room temperature until it is visibly dry (max. 60 min). The spatula used to distribute the inocula should first be used on a blank sample, to avoid test field 1 becoming contaminated with an insufficient quantity of inoculum.

19.6a Method for testing a disinfectant solution applied by wiping

After the inoculum has dried, the wiping procedure is conducted using the standard wiping cloth (➡ *19.4.2*), which had been impregnated 30 min ± 5 min beforehand with 16 ml of the test product. The wiping procedure with a wipe is conducted using a unitary weight (➡ *Figure 19.2*). The soaked wipes shall be weighed immediately before and after the wiping procedure, in order to be able to determine the quantity of test product liquid released onto the surface. The wiping procedure begins at test field 1 to test field 4 and back again to test field 1 (➡ *Figure 19.3*).

Carry out the wiping process across 1 – 2 – 3 – 4 within one second, immediately turn around and go back in the reverse direction (from 4 to 1) within another second. This way the test fields 1 – 4 are wiped twice and the entire test surface is wetted. Immediately after the wiping procedure is completed, start the stop watch and store the (test) surface at room temperature for the applicable contact time (t).

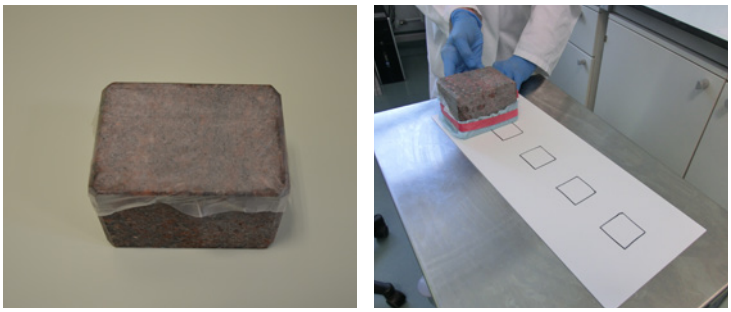

Abbildung 19.2: The bottom of the unitary weight is covered with Parafilm. The cloth, folded once, is placed on the protected area with Parafilm and fixed with a rubber. The weight is pushed by hand across the test surfaces without applying additional pressure.

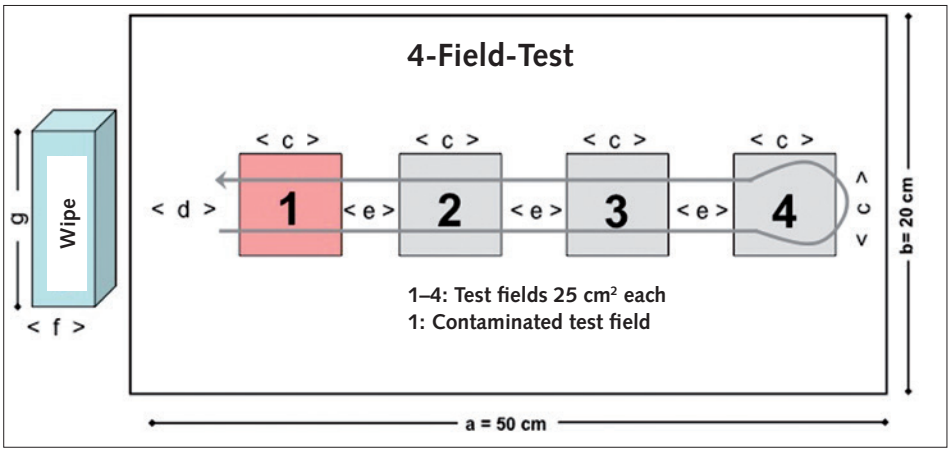

Figure 19.3: Diagram of the 4-field test. Test surface (20 x 50 cm) with 4 test fields (5 x 5 cm) and stipulated wiping route of the wiping cloth. a = 50 cm, b = 20 cm, c = 5 cm, d = 10 cm, e = 5 cm, dimensions of the unitary weight f x g at least 8.6 cm x 12.1 cm.

19.6b Method for testing the combination disinfectant solution applied with specified wiping cloth

After the inoculum has dried, the wiping procedure is conducted using the wiping cloth (wipe) provided by the manufacturer. In impregnated wipe systems the specified wiping cloth is impregnated 30 min ± 5 min prior to the wiping procedure test with the quantity of test product as prescribed by the manufacturer. In ready-to-use wipe systems the ready-to-use wipes are used directly as instructed by the manufacturer after opening the packaging. The manufacturer must specify how the wipes are to be used (e.g. the number of ply). That information must also be recorded in the test report. The wiping procedure takes place in the same way as described for method ➡ *Method 19.6a.*

To verify the claimed use period after preparing / opening the impregnated wipe systems / ready-to-use wipe systems, the second test run is conducted with the wipes at the end of the claimed use period (➡ *19A Requirements*). The WSH control 19.10.2 is performed when testing according to *19.1.6b* with the standard wiping cloth (➡ *19.4.2*).

The test wipes must have a minimum size of 9.5 cm x 13 cm.

19.7 Recovery of the test organisms from test field 1–4

Verification of recoverable cfu from each of the test fields 1–4 is done with the swabbing procedure. The entire test field 1 is wiped with a cotton swab moistened with neutraliser (➡ *Appendix A1.7, validated within the quantitative suspension test VAH method 18*) in a horizontal, vertical and diagonal direction. This recovery process is repeated using the same swab after it has been washed out in neutraliser. Subsequently, the lower half of the swab is transferred to the neutraliser test tube containing 5 ml neutraliser by cutting

it off at the edge of the neutraliser test tube. The recovery process is repeated once on the same test field with a second, dry cotton swab until the test field is visibly dry. The lower half of the swab is likewise transferred to the same neutraliser test tube and mixed. The recovery process takes roughly 1 min per test field. The two cotton swabs used are combined in 5 ml neutraliser per test field. Recovery from test fields 2 to 4 takes place in the same way (➡ *Diagram D6*). The parts of the swab transferred later to the neutraliser must not be touched.

Mix again thoroughly for approx. 1 min before further processing the neutraliser test tubes with swabs to assure optimal resuspension of the remaining test organisms from the swabs.

After 5 min ± 10 s neutralisation time, 1 ml aliquots from the respective test neutralisation mixture (direct plating) are poured in duplicate into BHIYT-L agar or, if using the spread plate method, spread onto pre-reduced plates. For test field 1 1 ml aliquots from the 10^{-1} dilution in neutraliser are poured additionally in duplicate or, if using the spread plate method, spread onto pre-reduced plates (➡ *Diagram D9*).

➡ see Annex **A1.7**

➡ see Diagram **D9**, Annex D

Method 19 was validated with the pour plate method. The spread plate method is also a recognized method for *Clostridium difficile*. If the spread plate method is used, the BHIYT-L agar plates must be pre-reduced overnight for at least 12 h at 36 °C ± 1 °C in an anaerobic jar.

➡ for incubation see **19.8**

➡ for calculation and presentation of the results see **19.9**

19.8 Inkubation

The colonies of viable spores are counted after 5 d incubation time at 36 °C ± 1 °C under anaerobic conditions.

19.9 Evaluation

For test field 1, culture media (plates) with colony numbers between 14 and 330 cfu are counted.

For test fields 2 to 4, culture media with colony numbers between 1 and 330 cfu are counted.

cfu_1: Number of cfu from 2 plates of 1 ml on test field 1
cfu_2: Number of cfu from 2 plates of 1 ml on test field 2
cfu_3: Number of cfu from 2 plates of 1 ml on test field 33
cfu_4: Number of cfu from 2 plates of 1 ml on test field 4

cfu_{T1}: Number of cfu per 25 cm^2 (test field 1) (corresponds to cfu_1 x 5)
cfu_{T2}: Number of cfu per 25 cm^2 (test field 2) (corresponds to cfu_2 x 5)
cfu_{T3}: Number of cfu per 25 cm^2 (test field 3) (corresponds to cfu_3 x 5)
cfu_{T4}: Number of cfu per 25 cm^2 (test field 4) (corresponds to cfu_4 x 5))

cfu_{To}: Number of cfu per ml on control field T_0 x 5 (➡ *19.10.1*)

cfu_{TT}: Number of cfu per ml on control field T_t x 5 (➡ *19.10.1*)

R: Reduction on test field 1

RC: Residual contamination on test field 1

AF: Accumulation test fields 2–4

The reduction (R) is calculated according to the following formula:

$lg\ R = lg\ (cfu_{Tt}) - lg\ (cfu_{T1})$

$lg\ RC = lg\ (cfu_{T1})$

$AF2–4 = [(cfu_{T2} + cfu_{T3} + cfu_{T4})) / 3]$

19.10 Validation

19.10.1 Recovery control after drying (T_0, T_t)

To quantify the recovery without any chemical or mechanical influence (drying control), two control test surfaces measuring 5 x 5 cm (T_0 and T_t) are contaminated in the same way as test field 1 (➡ *19.5*), parallel to the contamination of test field 1 on a separate test surface (at least 7 x 13 cm).

The recovery from test field T_0 takes place immediately after drying and before the wiping procedure on the contaminated test surfaces.

The recovery of the test organisms from test field T_t takes place after the contact time (T_t), in order to be able to quantify whether test organisms are inactivated during the contact time without treatment.

The recovery of the test organisms from test fields (T_0 and T_t) takes place by means of the swabbing procedure (➡ *19.7*). The entire test field, T_0 or T_t is wiped with a cotton swab moistened with neutraliser in a horizontal, vertical and diagonal direction. This recovery process is repeated using the same swab after it has been washed out in neutraliser. Subsequently, the lower, untouched, half of the swab is transferred to the neutraliser test tube. The recovery process is repeated once on the same test field with a second, dry cotton swab. The recovery process takes approx. 1 min per test field. The two cotton swabs used are combined in 5 ml neutraliser per test field (➡ *Diagram D9*). After the neutralisation time of 5 min ± 10 s, a 10^{-3} and 10^{-4} dilution in neutraliser (➡ *Annex A1.7*) is prepared and from this 0.1 ml is poured in duplicate into BHIYT-L agar or, if using the spread plate method, spread onto pre-reduced plates.

The colony forming units are determined for each test field (cfu/25 cm^2).

19.10.2 WSH control (Co1)

➡ for incubation see **19.8**

➡ for calculation and presentation of the results see **19.9**

To determine the number of cfu per 25 cm^2 without product exposure (Co1), contaminated (inoculated) surfaces are treated for each contact time with WSH + 0.1% polysorbate 80 in parallel instead of the product test solution. Impregnation of the standard wiping cloth (➡ *19.4.2*), the wiping procedure and recovery of the test organisms are performed as described in *19.6a* and *19.7*.

1 ml aliquots from the "test neutralisation mixture" are poured in duplicate as well as one 10^{-1} dilution in neutraliser (➡ *Annex A1.7*) into BHIYT-L agar or, if using the spread plate method, spread onto pre-reduced plates.

➡ see Diagram **D9**, Annex D

➡ for incubation see **19.8**

➡ for calculation and presentation of the results see **19.9**

Note: *In the WSH control (Co1) the mean count detected on test fields 2–4 should be ≥ 10 cfu/25 cm^2.*

19.10.3 Neutralisation control (Co2)

This control can be omitted if there are already convincing results from the quantitative suspension tests (➡ *Method 18*). If such results are not available, this control is conducted once before the actual tests.

A control is carried out to verify successful neutralisation by transferring 0.1 ml from the product test solution into 5 ml neutraliser (➡ *Annex A1.7*). After 5 min ± 10 s neutralisation time (preparations with contact times ≤10 min after 10 s ± 1 s), 0.5 ml of a 10^{-2} dilution of the cfu determination series(validation suspension) is added. After the longest contact time, 0.1 ml aliquots from a 10^{-1} dilution and a 10^{-2} dilution in neutraliser (➡ *Annex A1.7*) are poured into BHIYT-L agar or, if using the spread plate method, spread onto pre-reduced plates

➡ for incubation see **19.8**

➡ for calculation and presentation of the results see **19.9**

Note: If there is insufficient neutralisation in the test [(less than 1.5 x 10^3 cfu/ml (= 50 % of the validation suspension in Co3)], a different neutraliser must be chosen.

19.10.4 Verification of the non-toxicity of the neutraliser (Co3)

This control can be omitted if there are already convincing results from the quantitative suspension tests (➡ *Method 18*). If such results are not available, this control should be conducted once before the actual tests.

This control is carried out by transferring 0.5 ml of a 10^{-2} dilution of the cfu determination series (validation suspension) into 5 ml neutraliser (➡ *Annex A1.7*) After 30 min 1 ml aliquots are taken from both a 10^{-1} dilution and a 10^{-2} dilution in neutraliser (➡ *Annex A1.7*) and poured into BHIYT-L agar or, if using the spread plate method, spread onto pre-reduced plates.

➡ for incubation see **19.8**

➡ for calculation and presentation of the results see **19.9**

Note: If a toxic effect is identified in the test [less than 1.5 x 10^3 cfu/ml (= 50% of the validation suspension in Co3)], a different neutraliser must be chosen.

19A Requirements for efficacy testing for VAH certification

Surface disinfection with mechanical action – simulated-use test (4-field test) against *Clostridium difficile* spores

When assessing a surface disinfectant the following tests are obligatory:

Obligatory

– *Determination of the sporicidal activity against Clostridium difficile in the quantitative suspension test (Method 18 or prEN 17126 Test conditions ➡ Table 19.1*

– *Testing the sporicidal activity against Clostridium difficile simulated-use test (4-field test) with mechanical action (Method 19.1); Test conditions ➡ Tables 19.1 and 19.2*

The test product must meet the requirements set out in ➡ **Table 19.3** for spores of *Clostridium difficile* R027 under the specified conditions within the specified contact time at 20°C ± 1°C.

Table 19.1: Test conditions in the quantitative suspension test (Method 18) and under simulated-use conditions (Method 19).

Field of application	Test organisms	Interfering substance	Test concentrations[1]	Test temperature [°C]	Contact times (min)[2]
Surface disinfection	*C. difficile* spores (NCTC 13366)	clean and/ or dirty conditions	Use dilution	20 ± 1	1[3], 5, 15, 30, 60, 240

[1] In addition to the dilutions required for identification of efficacy (activity) range limits. The test concentrations employed should be used in steps not exceeding the factor 10. In the simulated-use test only the use dilution need be tested.

[2] A minimum of 3 contact times should be tested in the quantitative suspension test and a minimum of 2 contact times in the simulated-use test, while including the claimed contact times. In the test, the contact time which lies directly below the shortest claimed contact time should also be considered. This also applies when the test is performed corresponding to EN standards.

[3] If 5 min is the claimed contact time, 1 min must also be tested.

Table 19.2: Contact times to be selected for the individual test runs of the simulated-use test of surface disinfectants (Method 19).

Claimed contact time	1st Test run	2nd Test run
Surface disinfection with mechanical action (Method 19)		
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
240 min	60 min, 240 min	240 min

December 10, 2018, epub ahead of print (© mhp Verlag)

Table 19.3 Requirements in the quantitative suspension test (Method 18) and under simulated-use conditions (Method 19).

Activity spectrum	**Sporicidal activity against** *Clostridium difficile*	
Requirements	Quantitative suspension test (Method 18/ prEN 17126)	Surface disinfection with mechanical action (Method 19)
lg reduction	4 lg	Test field 1: 4 lg
Residual contamination		Test fields 2–4: mean ≤ 50 KBE/25 cm^2 Test fields 2–4 (WSH control): mean ≥ 10 KBE/25 cm^2
Interfering substance	clean and/or dirty conditions	
Test organisms	*Clostridium difficile* spores (NCTC 13366)	

The simulated-use tests should be performed in three runs each:

Testing according to 19a (with standard wiping cloth):

A Performance of controls (Co2, Co3);

B *1st test run*: One test surface per concentration-contact time-relation and per water control (Co1);

C *2nd test run*: Two test surfaces each per claimed concentration-contact time-relation and one test surface per water control (Co1).

Testing according to 19b (with specified wiping cloth):

A Performance of controls (Co2, Co3);

B *1st test run* – immediately after preparation / opening: one test surface per concentration-contact time-relation and per WSH control (Co1);

C *2nd test run* – immediately after preparation / opening or after claimed reuse period: two test surfaces each per claimed concentration-contact time-relation and one test surface per water control (Co1).

Reference

1. prEN 17126 (Entwurf). Chemische Desinfektionsmittel und Antiseptika – Quantitativer Suspensionsversuch zur Bestimmung der sporiziden Wirkung im humanmedizinischen Bereich – Prüfverfahren und Anforderungen (Phase 2, Stufe 1). Beuth-Verlag 2017.

** Update notification as of 16 January 2019: Meanwhile the phase 2/step 1 test was published:*

EN 17126. Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants in the medical area – Test method and requirements (phase 2, step 1).