Testing of the cleaning efficacy and the cleaning-disinfecting efficacy of washer-disinfectors for thermolabile endoscopes (EWD) by using flexible endoscopes

Mitteilung der DGKH

Im Rahmen der Revision der ISO/TS 15883-5:2005 (Reinigungs-Desinfektionsgeräte - Teil 5: Prüfanschmutzungen und -verfahren zum Nachweis der Reinigungswirkung; Deutsche Fassung CEN ISO/TS 15883-5:2005) ist es erforderlich, die derzeit im Annex I aufgeführte deutsche Testanschmutzung zur Prüfung von Reinigungs-Desinfektionsgeräten für thermolabile Endoskope in ein aktualisiertes ISO-Format zu überführen. Dieses wurde von Birgit Kampf, Jürgen Gebel, Heike Martiny und Markus Wehrl für die DGKH-Sektion „Reinigung und Desinfektion" ausgeführt. Die in Annex I nicht enthaltene Methode zur Überprüfung weiterer Endoskop-Kanäle ist in der Entwicklung und die Publikation wird zu einem späteren Zeitpunkt erfolgen.

Cleaning is of essential importance in the framework of reprocessing flexible endoscopes. Only properly cleaned endoscopes can be disinfected safely and provide an adequate hygienic status assuring the safety of the next patient.

Based on the instruction for reprocessing of the manufacturers of flexible endoscopes precleaning and manual cleaning need to be conducted prior to automated reprocessing in washer-disinfectors for flexible endoscopes (EWD). The requirements for the cleaning efficacy as well as for the complete process efficacy – as combined effect of cleaning and disinfection – of EWD have first been defined in ISO 15883-4 "Washer-Disinfectors: Requirements and tests for washer-disinfectors employing chemical disinfection for thermolabile endoscopes" in 2008 and extended in the revision of this standard in 2018.

A method based on references [1] and [2] to evaluate whether the EWD meets the requirements defined in the above mentioned standard is described and hereafter modified for using flexible endoscopes itself as test pieces.

A1 Principle

Tests on cleaning efficacy [3] and complete process efficacy [4] should be performed using test pieces (PCD) first, followed by tests using surrogate channel systems as described in the revision of the ISO 15883-4:2018, Annex H [5], prior to using soiled flexible endoscopes as described hereafter. Only after having met acceptance criteria for cleaning and complete process efficacy using PCD and surrogate channel systems, subsequent tests with soiled endoscopes are performed. Selection of endoscopes should represent instruments of all relevant type test groups.

For testing the cleaning efficacy on flexible endoscopes, the instrument channel is soiled with reactivated sheep blood. Testing the complete process efficacy is done using reactivated sheep blood containing test organisms.

The soiled endoscopes are loaded into the EWD to be tested. For the evaluation of the cleaning efficacy endoscopes are removed after the cleaning and the subsequent intermediate rinse. For the evaluation of the complete process efficacy endoscopes are removed after the final rinsing but before the drying. The endoscopes are removed from the EWD, inspected for visual cleanliness – especially at the channel openings/valve openings – and subsequently tested for residual protein content by using the modified OPA-method (see C1.5.2) to assess cleaning efficacy or tested for reduction of test organisms (see C1.6) to assess the efficacy of the complete process.

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B1 Materials

B1.1 Test soil

For testing the cleaning efficacy the test soil is a mixture of

- Sheep blood with 10 IE/ml heparin
- Protamine hydrochloride or protamine sulfate, applied to a final concentration of 15 IE/ml blood
- Saline (0.9 % NaCl solution)

For testing the complete process efficacy the test soil is a mixture of

- Sheep blood with 10 IE/ml heparin
- Protamine hydrochloride or protamine sulfate, applied to a final concentration of 15 IE/ml blood
- Saline (0.9 % NaCl solution)
- Suspension of selected test organism

Note: The usage of pooled sheep blood is recommended.

B1.2 Test pieces

- Suitable endoscopes based on type test groups according to ISO 15883-4:2018, annex H
- Suitable connectors/adapters, to connect each channel of the endoscope with the EWD

B1.3 Test organism

For testing the complete process efficacy (as combination of cleaning and disinfecting effect) the following test organism should be used:

- *E. faecium* (ATCC 6057, DSM 2146) In case a microorganism other than *E. faecium* proves to be more resistant in quantitative suspension tests according to EN 13727, EN 13624, EN 14348, EN 17126 or EN 14476 to the disinfection parameters of the EWD, all tests shall be performed using this specific microorganism instead of *E. faecium*.

Prepare a subculture by passaging *E. faecium* ATCC 6057 twice over CSL at (36 ± 1) °C for 24 h. Transfer 0.1 ml of this subculture onto CSA, plate with a Drigalski spatula and incubate for 72 h at (36 ± 1) °C. Approximately 10–15 petri dishes are required to prepare the test soil for one PCD. Remove the colonies from the agar surface using sterile saline solution. The suspensions of the rinsed petri dishes are collected in a beaker, whose bottom is covered with sterile glass beads.

Then homogenize the bacterial suspension by shaking for 10 minutes at 250 rpm. Centrifuge the suspension for 10 min at 3,000 g. Resuspend the test organisms by adding a small volume of saline and glass beads (approximately 0.25x of the volume of added saline). Shake for 1 minute at 500 rpm. An aliquot of the suspension is diluted in serial and plated out to determine the number of contained test organisms; the bacterial concentration shall yield at least 1 x 10^{11} CFU/ml.

Note: The number of subcultures shall not exceed three. For details regarding the maintenance of microbiological stock cultures, see EN 12353:2013-04.

B1.4 Microbiological media

- Saline solution (0.9 % NaCl)
- Casein peptone soy bean peptone broth (CSL)
- Casein peptone soy bean peptone agar (CSA)
- Kanamycin esculin azide agar (KAA-agar)
- If necessary, neutralizing media

B1.5 Equipment and consumables

- Balance with a resolution of ≤ 1 mg, calibrated
- Micro liter pipettes for diverse volumes, calibrated
- Pipette tips, diverse sizes
- 10 ml syringes (single-use)
- 20 ml syringes (single-use)
- 50 or 60 ml syringes (single-use)
- Centrifuge
- Centrifuge tubes 50 ml (e. g. Falcon™ tubes)
- Water bath with a temperature range up to 60 °C
- Membrane filtration system for filter sizes of 50 mm Ø
- Membrane filters, pore size 0.45 µm, Ø 50 mm
- Shaker
- Vortex mixer
- Glass beads, Ø 3–4 mm
- Beakers, diverse sizes
- Drigalski spatula
- Stop watch

B1.5.1 Equipment and consumables for the modified OPA-method

In addition to the equipment listed in B1.5 the following is needed:

- UV/VIS-photometer suitable for measurements at λ = 340 nm
- Cuvettes: quartz or single use plastic suitable for measurements at λ = 340 nm
- pH-meter, calibrated
- Volumetric flasks 100 ml, class A (e.g. from Carl Roth, Order-No: Y281.1)
- Volumetric pipettes 5 ml, class A or AS (e. g. from Carl Roth, Order-No: E976.1)
- Volumetric pipettes 2 ml, class A or AS (e. g. from Carl Roth, Order-No: E973.1)
- Reaction tubes, diverse

B1.5.2 Chemicals for the modified OPA-method

- *ortho*-phthaldialdehyde, p.a.-grad (e. g. Merck, Order-No. 1.11452.0005)
- Methanol, p.a.-grade (e.g. Merck, Order-No. 1.06009.1011)
- Disodium tetraborate, anhydrous, p.a. grade (e. g. Merck, Order-No. 1.06306.0250)
- 1 % SDS solution (w/w) in H_2O, adjusted with NaOH to pH = 11 (SDS, sodiumdodecylsulfate, grade for biochemical applications [e. g. Merck, Order-No. 1.12533.0050])
- 20 % SDS solution (w/w) in H_2O, pH value not adjusted
- 2-(dimethyl-amino) ethanethiolhydrochloride, 95 % (e. g. Aldrich, Order-No. D141003-25G)
- L-leucine, grade for biochemical applications (e. g. Merck, Order-No. 1.05360.0025)
- Bovine serum albumine (BSA), fraction V, purity ≥ 98 % (e. g. Carl Roth, Order-No. T844.2)
- H_2O, HPLC-grade (e. g. Merck, Order-No. 1.15333.2500)

C1 Procedure

C1.1. Preparation of the test soil for the evaluation of the cleaning efficacy

The heparinised sheep blood, the protamine solution and the saline are warmed up to room temperature and thoroughly mixed. The test soil for one endoscope/channel is made up of the following constituents:

- 11.4 ml heparinised sheep blood
- 0.42 ml saline (0.9 % NaCl solution)
- 0.18 ml protamine solution, applied to a final concentration of 15 IE/ml blood

One after the other, the components are transferred to a beaker, mixed thoroughly yet very carefully to prevent shear forces and a possible bubbling of the blood.

To determine the coagulation interval of the sheep blood, a stop watch is started right after the addition of the

protamine. Required coagulation time and room temperature are documented. After mixing the test soil an aliquot of 100 µl is taken and immediately diluted in 9.9 ml of a 1 % SDS solution, for a subsequent determination of the protein concentration of the test soil using the modified OPA-method according to [3].

Note: The complete coagulation has to be reached in less than 30 min.

C1.2 Preparation of the test soil for the evaluation of the complete process efficacy

The heparinised sheep blood, the protamine solution and the bacterial suspension are warmed up to room temperature and thoroughly mixed. The test soil for one endoscope/channel is made up of the following constituents:

- 11.4 ml heparinised sheep blood
- 0.18 ml protamine solution, applied to a final concentration of 15 IE/ml blood
- 0.42 ml bacterial suspension (B1.3)

For preparation follow the procedure of C1.1.

C1.3 Soiling of the endoscope

10 ml of the test soil are taken up with a syringe and injected into the instrument channel using suitable adapters (if applicable). After an incubation time of 30 seconds, 2 × 10 ml of air is injected into the same channel to blow out excessive soiling. Excessive soil that is expelled at the distal end of the endoscope is collected in a beaker of known weight. The amount of expelled soil is quantified using a balance. Based on the weight of expelled soil and taking into account residues in the beaker and syringe, the amount of soil residing in the channel is evaluated. Expelled soil is used to determine the time needed for coagulation. Therefore the beaker is swayed slightly. The start of the coagulation is recognized by a gel-like hardening of the surface, the period is assessed by using a stop watch. The time required for onset of coagulation, the room temperature and the calculated amount of soil loaded into the channel are documented. The soiled endoscope is incubated for one hour at room temperature, then patency of the soiled channel is checked by injecting 20 ml of air.

Note: Injection of fluids into channels should always be conducted in the same flow direction:

- Soiling
- EWD process
- Elution

C1.4 Placing the endoscope into the EWD chamber

For the measurement of the process efficacy, the soiled endoscope has to be put into the EWD according to the manufacturers Instruction for use (IFU) of the EWD by using the respective adapters/channel separators.

C1.5 Evaluation of the cleaning efficacy

C1.5.1 Elution of protein residues

For the quantification of the residual protein content in reprocessed endoscopes as well as in untreated positive and negative controls, residues have to be eluted. Therefore a defined volume of 1 % SDS solution (pH=11) is taken up with a suitable syringe and injected into the instrument channel. The amount of injected SDS solution should equal 80 % of the inner channel volume. The injection is conducted in the same direction as the soiling of the endoscope channel and the preceding flushing during reprocessing in EWD. The SDS solution is collected in a beaker and then pulled back into the channel to allow for an incubating over 30 minutes at room temperature. Afterwards, the SDS solution is expelled into the beaker and pulled up into the channel again. This rinsing process is repeated once more. Finally, the SDS solution is expelled. To drain the channel completely 2 × 50 ml of air are injected to remove residual SDS-solution. The protein content of this eluate is quantified by the modified OPA-method, described elsewhere [3].

Note: As there are strong indications that recovery rates could be improved, there are new elution methods currently under practical investigation that will be published when affirmed to be suitable.

C1.5.2 OPA-method

The following descriptions refer to a sample volume of 1 ml which might have to be modified with respect to the available equipment. Before usage all solutions have to be warmed up to room temperature. The measuring wavelength is $\lambda = 340$ nm. The measurement of each sample is performed in triplicate. For subsequent calculations the average values are used. For further details refer to [3].

C.1.6 Evaluation of the complete process efficacy

C.1.6.1 Elution of the test organism

Reprocessed endoscopes are analyzed by the elution of the soiled instrument channel as well as other non-soiled channels. Elution must be done with a neutralization medium. The selection of suitable neutralization media is based on information and recommendations provided by the disinfectant manufacturer or can be chosen using the VAH-Method 7 [6].

For the elution of the test organisms from each channel neutralization medium of twice the internal volume of the instrument channel is taken up with a syringe for each channel. First the non-soiled channels are flushed by connecting the syringe to the respective channel port or suitable adapter. The soiled instrument channel is eluted as the last one. The medium is injected in the same direction as the preceding flushing in the EWD and the test soil (instrument channel). Each volume of neutralization medium is quantitatively removed by the subsequent injection of each 2 x 50 ml air. The eluates are separately collected in sterile beakers (e. g. 200 ml volume). The bottom of the beaker is completely covered with glass beads to allow homogenization of the eluate by shaking for 3 minutes at 250 rpm. The eluates are membrane filtered. To transfer the eluate quantitatively from the beaker to the filter, 150 ml of sterile saline is filled into the beaker, which is briefly shaken and this saline is filtered as well. The membrane filters are transferred onto KAA-agar and incubated at (36 ± 1 °C). The total number of colonies (CFU_M) is counted after 24 hours, a check-up follows after 48 hours, all results are documented.

The quantification of high numbers of test organisms is essential for the evaluation of non-processed positive controls (CFU_C) or for the optimization of processes with low RF-values and respectively high numbers of residual test organisms (CFU_D). Therefore an aliquot of 1 ml of the collected eluate is taken and plated on KAA-agar medium after serial dilution.

The quantification of test organisms by plating is done in duplicate. For the quantification of colony forming units (CFU), samples are diluted serially and aliquots of 100 µl are plated on KAA-agar. The petri dishes are incubated at

(36 ± 1 °C), a growth of test organisms is indicated by formation of colonies with black color.

Note: As there are strong indications that recovery rates could be improved, there are new elution methods currently under practical investigation that will be published when affirmed to be suitable.

D1 Calculation

D1.1 Cleaning Efficacy

D.1.1.1 Visual Cleanliness

After the EWD process, the outer surface of the endoscope has to been assessed for visual cleanliness.

D.1.1.2 Evaluation of residual protein (cleaning efficacy)

The recovery rate (RR) of proteins for test pieces has the following correlation: RR = 100 • RPC/(WTS • PC)

RPC: eluted residual protein content of the test piece in µg
WTS: gravimetrically determined weight of test soil in a test piece given in g, that equals the volume in ml for an assumed density of 1.0 g in ml^{-1}
PC: protein concentration of the used test soil in µg ml^{-1}

D1.2 Efficacy of Cleaning & Disinfection (complete process)

D.1.2.1 Visual Cleanliness

After the EWD process, the outer surface of the endoscope has to been assessed for visual cleanliness.

D.1.2.2 Evaluation of residual microorganisms (cleaning & disinfection efficacy)

For evaluation of the complete process efficacy the endoscope channel has to contain ≥ 1 x 10^9 CFU (channel)$^{-1}$. The initial amount of test organisms in a channel ($CFU_{Channel}$) is calculated as follows: $CFU_{Channel} = WTS * CFU_T$

WTS: gravimetrically determined weight of test soil in an endoscope channel given in [g], that equals the volume in [ml] for an assumed density of 1.0 g ml^{-1}

CFU_T: number of test organisms in the test soil ml^{-1}

The microbial reduction factor (RF-value) for every endoscope channel is calculated as follows: $RF = \log (CFU_{Channel} / (CFU_M + CFU_D)$

CFU_M: number of eluted and membrane filtered test organisms filter $^{-1}$
CFU_D: number of eluted test organisms quantified by culturing of serial dilutions and corrected for the overall volume of neutralization medium.

NOTE 1: The microbial reduction factor (often termed RF-value) is the extent to which the bioburden is reduced in tenfold increments. It is expressed as $\log_{10}$ according to definition 3.174 in ISO 11139.

NOTE 2: Recovery rates for test organisms in non-processed PCD are between 0.1 and 2.0 %. Higher recovery rates using the described method indicate for an incomplete coagulation of the sheep blood or other failures.

D2 Acceptance criteria

After removal of the endoscope from an EWD the endoscope must not show any visually detectable residues of the soiling.

For cleaning efficacy, the analysis of protein residues in EWD processed endoscopes provides the following information:

- the level of total residual protein content in processed endoscopes/ channels
- the reduction factor (RF) for the removal of the test soil after treatment in an EWD.

As acceptance criterium the residual protein content of the soiled channel should be < 3 µg/cm^2 inner channel surface.

For complete process efficacy (cleaning & disinfection), the analysis of remaining test organisms in EWD processed endoscopes provides the following information:

- the total number of residual test organisms in processed endoscopes/ channels
- the microbial reduction factor (RF-value) for the reduction of the test organisms after treatment in an EWD.

The following acceptance criteria apply:

- Reduction of the test organisms in the soiled endoscope channel RF ≥ 9
- No detection of test organisms in other (non-soiled) endoscope channels
- Test organisms not detectable in the final rinse water.

References

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[2] Testing and evaluating the cleaning and disinfection efficacy of washer-disinfectors and disinfection automats. HygMed 2001; 26: 524.

[3] Wehrl M, Kircheis U: Method for Testing the Cleaning Efficacy of Washer-Disinfectors for Flexible Endoscopes. Central Service 2011; 19 (5): 357-361

[4] Kircheis U, Wehrl M: Method for Testing the Cleaning and Disinfecting Efficacy of Washer-Disinfectors for Flexible Endoscopes. Central Service 2012; 20 (4): 245-249

[5] ISO 15883-4:2018, Washer-Disinfectors – Part 4: Requirements and tests for washer-disinfectors employing chemical disinfection for thermolabile endoscopes.

[6] Desinfektionsmittel-Kommission im VAH: Anforderungen und Methoden zur VAH-Zertifizierung chemischer Desinfektionsverfahren. 1. Auflage, 2. April 2015, mhp-Verlag, Wiesbaden, 2015; also available as e-book (Requirements and Methods for VAH certification of chemical disinfection procedures) from mhp Verlag at https://bit.ly/2GYkmpG