# Investigation of the cleaning efficacy of washer-disinfectors for thermolabile endoscopes

## Multicentre trial on using a tube model with protein detection

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Following the publication of the Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Thermolabile Endoscopes (currently not available in English), a multicentre trial was conducted on behalf of the German Society of Endoscopy Nurses and Assistants (DEGEA) in which, pursuant to Annex 8 of the guideline, test pieces in the form of a tube model were investigated with protein detection. This trial assessed testing and evaluation of the cleaning efficacy at the time of validation as well as the specifications for the acceptance criteria in 17 endoscopy units in terms of their suitability under practical conditions. The cleaning processes were tested in ten washer-disinfector models using alternating combinations of seven different detergents. The results of the multicentre trial demonstrated that the majority of the processes tested met both acceptance criteria, i.e. optical cleanliness and residual protein amount in the test pieces. However, faulty connections, one technical defect as well as inadequate cleaning efficacy of washer-disinfector processes were also detected. In the multicentre trial the test pieces and combination of acceptance criteria proved to be suitable for verifying the cleaning efficacy during performance qualification at the time of validation.

KEY WORDS
- multicentre trial
- cleaning efficacy
- washer-disinfectors
- thermolabile endoscopes
- test pieces
- protein determination
- OPA method

## Introduction

While compiling a Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Thermolabile Endoscopes (1), a working group was set up to devise test methods. Based on the existing method pursuant to ISO/TS 15883-5 Annex I (2), the aim was to modify and streamline this so as to devise models for testing the minimum cleaning efficacy as well as the entire process. These investigations led to the development of a test piece model for determination of the cleaning efficacy based on quantification of the key protein parameter (1, 3). The aim now was to use this test piece for performance qualification of the cleaning process of washer-disinfectors for thermolabile endoscopes (EWD) at the time of validation. To test the suitability and practicability of the new test pieces, a practical test was organized by the German Society of Endoscopy Nurses and Assistants (DEGEA) in which virtually all EWD models used in the healthcare services in Germany were tested in combination with detergents from different manufacturers.

This present article now presents the results of the multicentre trial. It also discusses the findings of visual assessment of test pieces as well as the values obtained for the residual protein amount in test pieces with reference to the acceptance criteria specified in the Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Thermolabile Endoscopes for the guide value, alarm value and limit value.

## Materials and Methods

### *Washer-disinfectors tested*

The following washer-disinfectors were tested within the framework of the multicentre trial:

AdaptaScope (Wassenburg), ETD2 (Olympus), ETD2 Plus (Olympus), ETD3 (Olympus), ETD3 Plus (Olympus), Innova E3 (BHT), Innova 2000 (BHT), SME 2000 (BHT), WD 425E (Belimed), WD 430 (Belimed).

### *Detergents tested*

One of the following detergents was investigated in the test washer-disinfectors: ETD Cleaner (Olympus), EndoDet (Olympus), Korsolex Endo-Cleaner (Bode), Mucapur-ER plus (Merz), neodisher MediClean forte (Dr. Weigert), Thermoton NR (Dr. Schumacher), Thermosept ER (Schülke+).

### *Conduct of the multicentre trial*

To minimize variations with respect to production of the test pieces, tests in the endoscope washer-disinfector (EWD) as well as evaluation of test pieces were carried out on behalf of the DEGEA by a single test laboratory, wfk – Institut für Angewandte Forschung GmbH, Krefeld, Germany.

The test pieces were produced in accordance with the method published as Annex 8 of the Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Thermolabile Endoscopes (3). That method rules

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out blockage caused by coagulated blood, while assuring patency as a precondition for use of the test pieces. The test pieces were freshly prepared for each test and used 4 – 30 hours after production. They were stored at approx. 4 °C, and transported to the user in a cooling box without any additional temperature regulation.

In addition to the test pieces, three negative controls (test pieces without test soil) and three positive controls (test pieces with test soil) were produced and evaluated as batch controls for each test. Some of the negative controls served as batch controls for the PTFE tubular material used to produce the test pieces, in order to ascertain whether any protein amount present in the tubular material had an impact on the results. The positive controls were not exposed to any cleaning process and were used to elucidate the baseline protein amount per test piece. Two to three additional negative controls were transported under refrigerant conditions together with the test pieces and used in the test process.

The test pieces were connected in the EWD using, whenever necessary, corresponding adapters provided by the EWD manufacturers. The test pieces were positioned in the washer-disinfectors in such a way as to ensure that they were flushed in the EWD in the same direction as when soiling. For process control purposes, pressure and temperature loggers (from the firm Ebro) were connected to an unoccupied port in the EWD and the detergent dosage quantities were gravimetrically measured.

The reprocessing processes investigated were those routinely used in the respective endoscopy units for reprocessing purposes. Two test pieces and a negative control were used concurrently for each test. Each test was repeated twice, hence six contaminated test pieces were tested in the EWD for each process. The reprocessing process was interrupted before the start of the disinfection phase since the focus of testing was confined to the cleaning efficacy of the cleaning phase.

### *Residual protein determination by means of the OPA method*

The test pieces as well as the negative and positive controls were eluted with 5 ml 1 % SDS (sodium dodecyl sulfate) solution (pH 11) in situ immediately after being used in the EWD, as described in Annex 8 (1). Eluates were transported under refrigerant conditions to the wfk laboratory, where they were stored for a maximum of 72 h at approx. 4 °C for further analysis in individual cases. Eluates were measured and protein content quantified in accordance with the provisions of Annex 8.

### *Detection limit of method*

Based on preliminary tests, the minimum amount of protein per test piece which could be determined with adequate precision was identified as being 50 µg protein/test piece (corresponding to 10 µg per ml eluate).

### *Evaluation of the multicentre trial*

Pursuant to the Guideline for Validation of Automated Cleaning and Disinfection Processes for Reprocessing Thermolabile Endoscopes (1), the test pieces were evaluated using two methods

– Visual assessment of optical cleanliness
– Determination of the residual protein amount in the test pieces

After opening the EWD, the test pieces were visually inspected. Five levels of residual soiling were identified:

(–) no residual soiling,
(+/–) one to two residual coagula,
(+) three to ten residual coagula,
(++) moderate residual soiling and
(+++) heavy residual soiling.

The following acceptance criteria were applied to assess the residual protein amount in test pieces (1):

Guide value: ≤ 800 µg protein/test piece
Alarm range: > 800 to ≤ 1600 µg protein/test piece
Limit value: > 1600 µg protein/test piece

The test pieces used as either negative or positive controls were likewise eluted and the residual protein amount was determined.

## Results

In the course of the multicentre trial, 18 cleaning processes were investigated in washer-disinfectors of different models and year of manufacture in combination with seven detergents from different manufacturers in 17 endoscopy departments. Performance qualification was repeated in its entirety for three processes (process 4W, 5W, 9W).

Since for each process tested, two test pieces were used, and in the majority of processes one negative control was employed per reprocessing cycle and altogether three reprocessing cycles were investigated, a total of 126 test pieces and 55 negative controls were used.

### *Tubular material batch controls*

Eight further negative controls were employed as batch controls for the tubular material used to make the test pieces.

Protein amounts below the limit of determination (LOD) of 50 µg protein/test piece were measured in the eluate of all batch controls. Hence, none of the batches of tubular material used to produce the test pieces contained any protein, or only amounts below the limit of determination which did not affect the test results.

### *Controls used in the process*

Likewise, the majority of the negative controls used in the process yielded values below the limit of determination. For a few negative controls values of up to 85 µg protein were measured in the eluate. Hence it can be concluded that the cleaning solution gave rise to only very little residual contamination of test pieces and, as such, essentially did not have any impact on the protein determination test results in the test pieces.

In the eluates of 60 positive controls, average protein amounts of 67,468 µg per test piece were detected. Therefore a maximum reduction of the protein amount of between three and four $\log_{10}$ levels can be detected with the test pieces used here.

### *Visual cleanliness*

Qualitative visual assessment of the 126 test pieces cleaned in the EWD revealed that 78 were optically clean, 28 showed slight visible soiling and 20 harboured heavy to very heavy blood residues (Table 1).

### *Quantitative protein determination*

Evaluation of quantitative determination of the protein amount in the test piece eluates showed that in the majority of processes investigated the mean value of the six test pieces used and the individual values were below the guide value of ≤ 800 µg protein/test piece stipulated in the Guideline (1) (Figure 1). In two processes (process 2 and 15), the mean values were in the region of the guide value, although, with regard to the individual test piece values, values that were either above or below the guide value were measured. In one process (process 10), the mean value was within the alarm range.

**Table 1: Visual assessment of test pieces (TP)**
Legend: * test piece became detached from adapter during the programme cycle
W: Repeat test
(–) no residual soiling
(+/–) one to two residual coagula
(+) three to ten residual coagula
(++) moderate residual soiling
(+++) heavy residual soiling

| Process | TP 1 | TP 2 | TP 3 | TP 4 | TP 5 | TP 6 |
|---|---|---|---|---|---|---|
| 1 | (–) | (–) | (–) | (–) | (–) | (–) |
| 2 | (+) | (+) | (+/–) | (+) | (+) | (++) |
| 3 | (+/–) | (+/–) | (+/–) | (–) | (–) | (+) |
| 4 | (+) | (++) | (+) | (+) | (++) | (++) |
| 4W | (+++)* | (+) | (+/–) | (+) | (+) | (++) |
| 5 | (+++) | (+++) | (+++) | (+++) | (+++) | (+++) |
| 5W | (–) | (–) | (–) | (–) | (–) | (–) |
| 6 | (–) | (–) | (–) | (–) | (–) | (–) |
| 7 | (–) | (–) | (–) | (–) | (–) | (–) |
| 8 | (–) | (–) | (–) | (–) | (–) | (–) |
| 9 | (+++) | (+++) | (+++) | (+++) | (+++) | (+++) |
| 9W | (–) | (–) | (–) | (–) | (–) | (–) |
| 10 | (+) | (+++) | (+) | (+/–) | (+) | (+) |
| 11 | (+/–) | (–) | (–) | (–) | (+/–) | (–) |
| 12 | (–) | (–) | (–) | (–) | (–) | (–) |
| 13 | (–) | (–) | (–) | (–) | (–) | (–) |
| 14 | (–) | (–) | (–) | (–) | (–) | (–) |
| 15 | (+) | (+) | (+) | (+) | (++) | (+) |
| 16 | (–) | (–) | (–) | (–) | (–) | (–) |
| 17 | (–) | (–) | (–) | (–) | (–) | (–) |
| 18 | (–) | (–) | (–) | (–) | (–) | (–) |

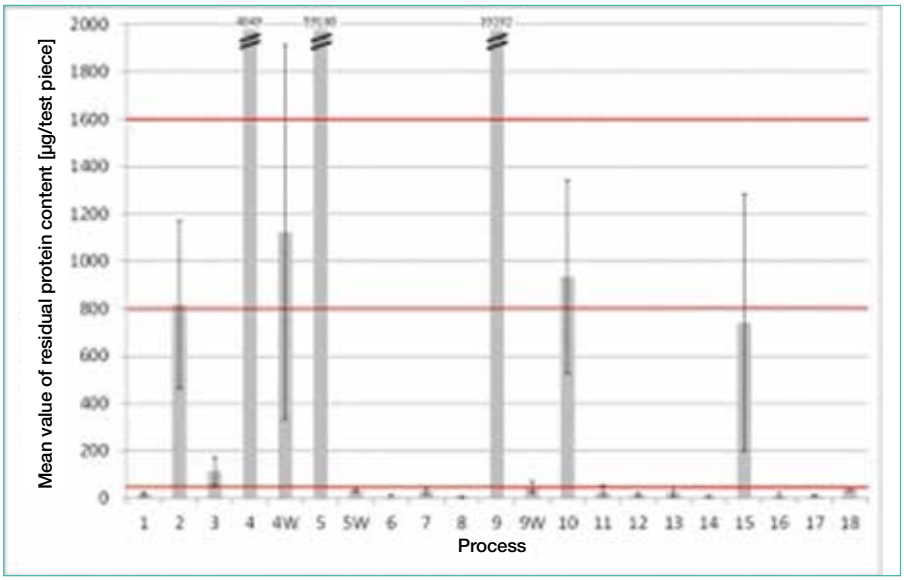


**Fig. 1: Mean residual protein amount per process in µg protein/test piece and standard deviation of test pieces used per process (n= 6)**
Legend: Lower red line: Limit of determination (50 µg protein/test piece)
Middle red line: Guide value (800 µg protein/test piece)
Upper red line: Limit value (1600 µg protein/test piece)

In processes 4, 5 and 9, repeat tests were conducted since residual protein amounts above the limit value of > 1600 µg protein/test piece were detected. In process 5, the EWD had not identified the test piece as an endoscope surrogate in the first performance qualification and, hence, did not rinse it. After modification of this connection, the test was repeated (process 5W) and in the test piece eluate protein amounts < 50 µg were detected. In process 9, one technical test of the EWD revealed that its circulation pump was defective. Here, too, very good cleaning results were obtained in the repeated performance qualification after elimination of the technical defect (process 9W).

Only in process 4, in the initial performance qualification as well as in a repeat test (process 4W) following technical investigation, were no results below the guide value obtained.

Evaluation of both qualitative visual assessment and of quantitative determination of the residual protein amounts in the test pieces revealed that, while taking account of the acceptance criteria specified in the Guideline, 12 of the 18 processes met both the qualitative and quantitative requirements and successfully passed performance qualification (Table 2). In two processes (process 3 and 11), the residual protein amounts in the test pieces were below the guide value of ≤ 800 µg, however, visual assessment of some test pieces revealed one to two residual coagula or three to ten residual coagula. Hence, the requisite optical cleanliness of test pieces was not assured and, accordingly, performance qualification not passed in terms of cleaning.

In the course of these tests, graduated assessment of visual inspections was introduced and applied. This assessment scheme correlates to a large extent with the values obtained in the tests for the residual protein amount in test pieces (Table 3).

## Discussion

The aim of the multicentre trial was to investigate a new test model pursuant to Annex 8 of the Guideline, while using a tubular test piece with protein detection (3), to identify the minimum cleaning efficacy of EWDs. Another aim was to investigate the acceptance criteria specified by the Guideline (1) in a large number of automated processes used to reprocess thermolabile endoscopes in different models of washer-

**Table 2: Assessment of 18 processes based on the acceptance criteria in the Guideline (1)**
Legend: W = Repeat test

| Process | Visual assessment | Residual protein amount | Overall assessment |
|---|---|---|---|
| 1 | passed | smaller than acceptance value | passed |
| 2 | failed | alarm range | repeat needed |
| 3 | failed | smaller than acceptance value | repeat needed |
| 4 | failed | greater than limit value | repeat needed |
| 4W | failed | greater than limit value | failed |
| 5 | failed | greater than limit value | repeat needed |
| 5W | passed | smaller than acceptance value | passed |
| 6 | passed | smaller than acceptance value | passed |
| 7 | passed | smaller than acceptance value | passed |
| 8 | passed | smaller than acceptance value | passed |
| 9 | failed | greater than limit value | repeat needed |
| 9W | passed | smaller than acceptance value | passed |
| 10 | failed | alarm range | repeat needed |
| 11 | failed | smaller than acceptance value | repeat needed |
| 12 | passed | smaller than acceptance value | passed |
| 13 | passed | smaller than acceptance value | passed |
| 14 | passed | smaller than acceptance value | passed |
| 15 | Failed | alarm range | repeat needed |
| 16 | passed | smaller than acceptance value | passed |
| 17 | passed | smaller than acceptance value | passed |
| 18 | passed | smaller than acceptance value | passed |

**Table 3: Relationship between visual assessment results and residual protein amounts detected for 126 test pieces**

| Visual assessment | Number of test pieces | Residual protein amount per test piece |
|---|---|---|
| (–) | 78 | < 100 µg |
| (+/–) | 8 | 100 µg to 500 µg |
| (+) | 20 | 500 µg to 900 µg |
| (++) | 6 | 900 µg to 6500 µg |
| (+++) | 14 | > 6500 µg |

disinfectors, from different manufactures and using varying processing times, in combination with detergents of different compositions and manufacture. This selection of test processes was thought to essentially take account of all combinations of EWDs and detergents currently used in Germany. The aim was to ascertain whether the test model lent itself to use in the various EWD makes and whether the acceptance criteria posed too great or too little a challenge for the minimum cleaning efficacy with regard to validation of EWDs. The test model cannot, and should not, serve as a substitute for the essentially more comprehensive cleaning efficacy tests conducted within the scope of the EWD type test.

The multicentre trial demonstrated that the test piece model did indeed lend itself to use in all EWDs with the adapters supplied by the manufacturers and yielded evaluable results. Furthermore, the multicentre trial revealed that the majority of processes investigated passed the test with respect to the two criteria, i. e. optical cleanliness and residual protein amount in the test pieces. In two processes technical problems were noted during testing (faulty connection or defective circulation pump). Once these problems were eliminated, both processes met the acceptance criteria for cleaning efficacy. One process in an older make of EWD that had not undergone type testing did not pass a second test after technical assessment. This may be an exception. But it could also point to a general problem with older EWD models that had not been subjected to type testing, and which may not meet the cleaning efficacy requirements. In two other processes, values within the alarm range for the protein amount per test piece were obtained. Such processes should be tested again within the framework of validation after technical investigation of the EWD and, as applicable, establishing the origin of the problems.

In the Guideline (1) the qualitative acceptance criterion always stipulated is optical cleanliness. The relationship between visual cleanliness and microbiological cleanliness (reduction of the baseline colony count) in a hose test piece during cleaning has been discussed by Zühlsdorf et al (4). The present studies made an initial estimate of the relationship between optical cleanliness and the protein amount per test piece (Table 3). It was revealed that on using the test soil employed in the present tests, the test pieces were optically clean only when the protein amount per test piece was around < 100 µg, corresponding to around 1 µg/cm$^2$. That value is far below the value of 6.4 µg/cm$^2$ discussed by M.J. Alfa et al (5, 6), which was used in the Guideline (1) as a reference point for setting the guide value for quantitative determination of the protein amount per test piece. If this relationship between the qualitative and quantitative parameters is confirmed again, the acceptance criteria should also be discussed again, and adjusted if necessary.

In summary, it can be stated that

- the new test model and the acceptance criteria are suitable for investigating the majority of washer-disinfectors used in Germany for automated reprocessing of thermolabile endoscopes with regard to the cleaning efficacy during performance qualification at the time of validation,
- there exists a relationship between optical cleanliness and residual protein content,

- technical defects affecting cleaning efficacy can be detected,
- the tubular material pursuant to (3) used to produce the test pieces does not harbour any protein amounts that essentially affect the test results,
- the test detergents are endowed with sufficient soil-removing properties to ensure that recontamination of the test pieces has no significant effect on the test result and
- the test pieces should harbour at least 40,000 µg protein before the start of testing in order to demonstrate reduction of the protein amount by three to four $\log_{10}$ levels. ■

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