Control of the cleaning efficacy during performance qualification of EWD processes using test pieces according to Annex 8

Qualitative and quantitative results of a field investigation

M. Wehrl

The evaluation of the cleaning efficacy of automated reprocessing procedures for thermolabile flexible endoscopes (EWD processes) is carried out using test pieces according to Annex 8 of the currently applicable guideline. The quantitative acceptance criteria for cleaning efficacy given in the currently applicable guideline (2011) were defined with reference to the publication of Alfa M.J. *et al.*, 1999 and 2002. To investigate the residual protein content in test pieces that were reprocessed with state-of-the-art EWD processes in hospitals and medical practices, a field investigation was organized and conducted by the guideline group and validation laboratories. Participating validation laboratories collected anonymized result data of performance qualification tests that were conducted between April and September 2015. Results of the residual protein content of 2298 test pieces were provided which were applied for the qualification of 888 EWD processes. Visually recognizable soil residues were detected in 127 test pieces. The other 2171 test pieces were visually clean, 91.7% of these exhibited a residual protein content of ≤ 100 µg/test piece. 0.3% of the visually clean test pieces exceeded the current guide value of ≤ 800 µg protein/test piece, the limit value of > 1600 µg protein/test piece was exceeded by 0.0% of the test pieces. The results of this field investigation implicate a reduction of the acceptance criteria for the quantitative evaluation of the cleaning efficacy of EWD processes.

KEY WORDS
- thermolabile flexible endoscopes
- cleaning efficacy
- test pieces
- residual protein content
- validation
- acceptance criteria

Introduction

According to the German Medical Devices Operator Ordinance MPBetreibV [1], the reprocessing of thermolabile flexible endoscopes has to be achieved applying validated processes to assure that cleaning and disinfection results in the required reprocessing success. For the validation of automated cleaning and disinfection processes for thermolabile endoscopes (EWD processes) the respective guideline of DGKH, DEGEA, DGSV, DGVS and AKI together with manufacturers of washer-disinfectors and endoscopes was published [2].

For the performance qualification (PQ), the cleaning efficacy and the overall process efficacy is evaluated using tubular test pieces, amongst others. These test pieces consist of a PTFE tubing with a length of 200 cm and an inner diameter of 2 mm and therefore refer to the channel system of real instruments.

For the evaluation of the overall process efficacy (cleaning and disinfection combined) a test soil consisting of reactivated sheep blood mixed with the test organism *Enterococcus faecium* is used. This test piece model is described in Annex 9 [3] of the guideline and is based on the test pieces described in ISO/TS 15883-5, Annex I [4].

The testing of the cleaning efficacy is accomplished using the tubular test piece soiled with reactivated sheep blood. For the evaluation of the cleaning effect after the process steps pre-rinse, cleaning and intermediate rinse (aborting the program straight before the disinfection step), the residual test soil is assessed by visual inspection and the residual protein content quantified. Proteins serve as guide parameter for the applied test soil. According to Annex 8 the quantification of proteins is carried out using the modified OPA method (*ortho*-phthaldialdehyde method). The protein content is referred to the equivalent amount of BSA (bovine serum albumin, fraction V). The methods for both the preparation and for the evaluation of test pieces were established by the so called "method group" that was initiated by the guideline group. A detailed description is found in Annex 8 [5] of the guideline.

Prior to publication the functionality of the test piece model was surveyed by investigations that were executed at EWD manufacturers [6]. The suitability under real conditions was further investigated in a multicentre trial commissioned and coordinated by DEGEA [7]. 18 EWD processes in hospitals and doctors' practices, were surveyed to prove their function.

The acceptance criteria of the guideline [2] give the definition of the minimum requirements and are applied to evaluate the cleaning efficacy. The first acceptance criterion is the visual cleanliness of the test pieces (qualitative evaluation). For clean test pieces the second acceptance crite-

Dr. Markus Wehrl, wfk – Cleaning Technology Institute e.V., Campus Fichtenhain 11, 47807 Krefeld, Germany. E-mail: m.wehrl@wfk.de

rion, the evaluation of protein residues, applies (quantitative evaluation). The assessment is carried out with regard to: i) guide value ≤ 800 µg protein/test piece, ii) warning level > 800, ≤ 1600 µg protein/test piece and iii) limit value > 1600 µg protein/test piece. The minimum requirements are defined by a) achieving visual cleanliness and b) compliance with the guide value.

The guide value was defined with reference to the publication TIR30:2003 [8] of AAMI (Association for the Advancement of Medical Instrumentation). The latter specifies a protein-surface-value of < 6.4 µg protein cm^{-2}. This acceptance criterion is also referred to in the updated version of TIR30:2011 [9]. The value refers to the publications of Alfa *et al.* 1999 [10] and 2002 [11] and is based on the quantification of protein residues in the channel system of endoscopes (real instruments) after manual reprocessing. At the time the guideline was published this protein-surface-value was the only specification to be found in the literature for the achievable residual protein content in the channel system of endoscopes. The application of this value to the test piece model (inner surface 126 cm^2) resulted in the definition of the guide value of ≤ 800 µg protein/test piece. The warning level and the limit value were set in parallel to the "Guideline of DGKH, DGSV and AKI for the Validation and Routine Monitoring of Automated Cleaning and Disinfection Processes for Heat-Resistant Medical Devices" [12].

The guideline group together with validation laboratories initiated a field investigation to establish a database on residual protein amounts in Annex 8-test pieces that can be achieved in practice using current state-of-the-art automated reprocessing procedures. The following validation laboratories participated (in alphabetical order): Belimed Deutschland GmbH, represented by Frank Wohlgemuth; Cleanical GmbH, represented by Dr. med. Dipl.-Ing. Thomas W. Fengler; Olympus Deutschland GmbH, represented by Christian Roth and Valitech GmbH & Co KG, represented by Dipl.-Ing. (FH) Daniel Geyer. Moreover Hybeta GmbH, represented by Dirk Diedrich, participated in the data collection using an alternative type of test piece (data not shown).

Participating validation laboratories collected data of the residual protein content in Annex 8-test pieces over a six month period. The test pieces were applied in performance qualification (PQ) testing of EWD processes in endoscope facilities all over the country. The result data were provided in an anonymised way and give an overview on the cleaning efficacy of state-of-the-art EWD processes.

Materials and Methods

The four participating validation laboratories Belimed Deutschland GmbH, Cleanical GmbH, Olympus Deutschland GmbH and Valitech GmbH & Co KG (in alphabetical order) applied Annex 8-test pieces during performance qualification (PQ) testing to investigate the cleaning efficacy of EWD processes in respective facilities. Each tested process, or each tested EWD respectively, was consecutively numbered, to avoid duplicate assessment due to repetitive process runs. Test pieces were evaluated qualitatively (1. acceptance criterion: visual cleanliness) as well as quantitatively (2. acceptance criterion: residual protein content). The result data of Annex 8-test pieces applied during PQ testing in the period between April 1st and September 30th 2015 were collected and analysed.

Anonymised results were provided monthly by filling in an enquiry matrix. The results of each test piece in a single process run as well as in repetitive runs, if applicable, were documented.

Furthermore, process specific information as well as general information on the manner of test execution was documented and provided. Amongst others, the data set included the following information

- EWD with or without type testing
- First PQ testing or re-testing
- Type of connection of the test pieces
- Type of the test pieces
- Age of the applied test pieces
- Process step, when the test pieces were removed
- Place and time the visual inspection took place
- Visual inspection of test pieces and classification of the results into the categories
  i) 0 coagula
  ii) 1 – 2 coagula
  iii) 3 – 10 coagula
  iv) > 10 coagula
- Applied protein quantification method
- Residual protein content

The data provided by the respective validation laboratories were anonymised with regard to the identity of the laboratory and analysed uniformly. The data were subsequently discussed by the guideline group and the validation laboratories and released for publication.

Results

In total 2298 Annex 8-test pieces were applied to test the cleaning efficacy of 888 EWD. One to three process runs were tested. Usually each process run was tested applying two test pieces. Some runs were tested using three test pieces. 97.2% of the tested EWD were type-tested, whereas 2.8% were not type-tested. The spectrum of tested EWD comprised machines with pressure chamber as well as machines with separate channel connection and irrigation. 19.0% of the EWD-processes were tested for the first time, 81.0 % were re-qualified.

All validation laboratories indicated to use test pieces according to Annex 8 of the guideline. Three of the laboratories carried out the visual inspection of test pieces immediately after the removal in the respective endoscopic facility. One laboratory accomplished the visual inspection in their laboratory facilities. For the quantification of protein residues three of the validation laboratories applied the modified OPA-method according to Annex 8, the other laboratory used the BCA- (bicinchoninic acid) method.

Age of the test pieces

Test pieces applied for PQ testing exhibited a different age (number of days between preparation and application in the EWD). The majority of test pieces were one to six days old. A low portion of test pieces had a higher age (maximum 14 days). The distribution of the relative frequency is plotted in Fig. 1.

Qualitative evaluation

Of the 2298 test pieces in total, n = 127 (5.5%) showed visually detectable residues (coagula). The number of test pieces with 1 – 2 coagula amounted to n = 18, the residual protein content of these ranged between 0 – 300 µg/test piece. The number of test pieces with 3 – 10 coagula was n = 19 with a residual protein content ranging between 0 – 801 µg/test piece.

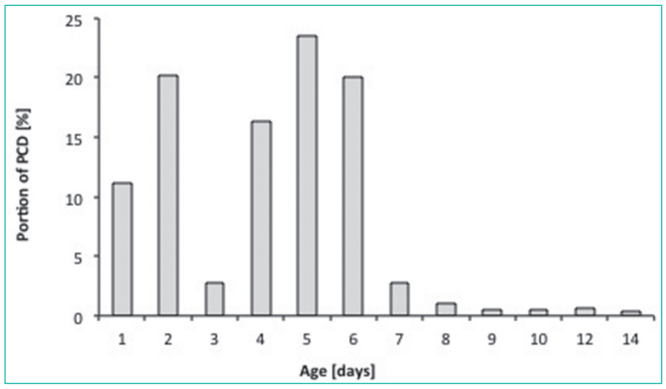

Fig. 1: Distribution of the relative frequency [%] of the age of test pieces (PCD) (number of days between preparation and application for PQ testing) (n = 2298 [≈100 %]).

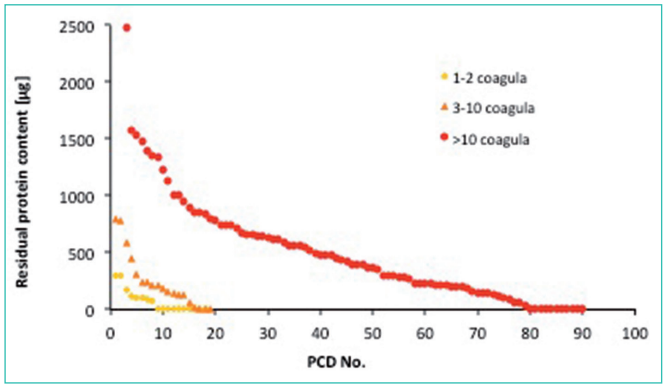

Fig. 2: Correlation between the residual protein content [µg test piece^{-1}] and visually detectable residues. Test pieces (PCDs) were classified into categories with regard to the number of contained coagula. Each dot represents one test piece. For optimized presentation two test pieces which contained > 10 coagula were not plotted, these exhibited a residual protein content of 3878 and 3684 µg, respectively (n = 127).

The number of test pieces with > 10 coagula was n = 90, protein residues amounted to 0 – 3878 µg/test piece. The results are summarized in Fig. 2.

A statistical delineation of the three categories of test pieces with their respective number of contained coagula with regard to minimum, maximum, median, lower and upper quartile is given as a boxplot in Fig. 3.

Test pieces containing visually detectable residues did not comply with the first acceptance criterion of the guideline. Subsequently these test pieces were rejected from further analysis.

Quantitative evaluation

The overall number of visually clean test pieces amounted to n = 2171, which were applied in 839 EWD. The arithmetic mean of the residual protein content was 29.9 µg/test piece, the sampling standard deviation was 98.4 µg/test piece (n = 2171). The number of test pieces the residual protein content could not be determined (reported as 0 µg/test piece) amounted to n = 1626 (74.9% of the 2171 test pieces).

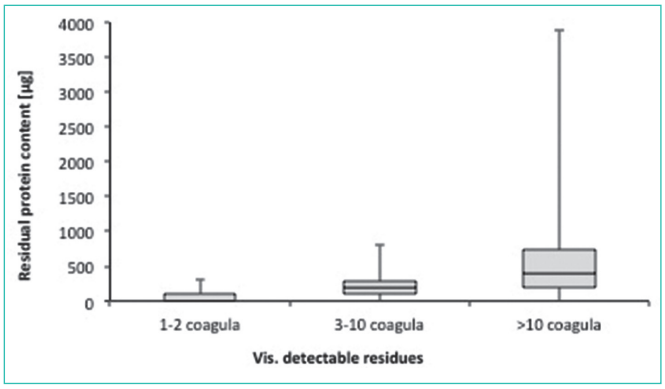

Fig. 3: The different categories of test pieces (1 – 2 coagula, 3 – 10 coagula, > 10 coagula) were analysed with regard to the measured residual protein content. The boxplot shows minimum, maximum, median, lower and upper quartile (n = 127).

For the analysis of the present data, the test pieces were categorized with regard to their residual protein content. Since the validation laboratories did not report information on the characteristics of their applied protein quantification methods and the achieved limits of quantification (the lowest protein content that can be quantified within the chosen limits of uncertainty), test pieces with a reported residual protein content of 0 µg/test piece (n = 1626) and such within 0 < × ≤ 100 µg/test piece (n = 364) were summarized to allow a standardized statistical analysis. Test pieces were classified into the following categories:

1. category: × ≤ 100 µg/test piece
2. category: 100 < × ≤ 200 µg/test piece
3. category: 200 < × ≤ 300 µg/test piece, etc.

The distribution of test pieces with regard to the frequency showed the following results: the number of test pieces with × ≤ 100 µg/test piece amounted to n = 1990, these with 100 < × ≤ 200 µg/test piece to n = 101. The results for the overall n = 2171 test pieces are summarized in Fig. 4. The number of test pieces that exceeded the current guide value of ≤ 800 µg protein/test piece was n = 7, the current limit value (> 1600 µg protein/test piece) was exceeded by not a single test piece.

The distribution of test pieces with regard to relative frequency showed the following results: 91.7% of all test pieces fell with in the category × ≤ 100 µg/test piece, 4.7% into the category 100 < × ≤ 200 µg/test piece, etc. The results are displayed in Fig. 5. The share of test pieces that exceeded the current guide value of ≤ 800 µg protein/test piece was 0.3%, the share that overshot the limit value (> 1600 µg protein/test piece) was 0.0%.

Different results regarding the cleaning efficacy may arise due to the vast number of different EWD in the respective endoscopic facilities, the different tested processes and the different process chemicals used. To investigate, if systematic differences between validation laboratories beyond the previously mentioned variations occurred, the relative frequency of results classified into the respective category was plotted for each validation laboratory.

The comparative diagram shows a good agreement of results with regards to the distribution of the measured residual protein content into the respective categories, see Fig. 6. The differences of the relative frequency for the category × ≤ 100 µg/test piece amounted to 6.4% between the four validation laboratories.

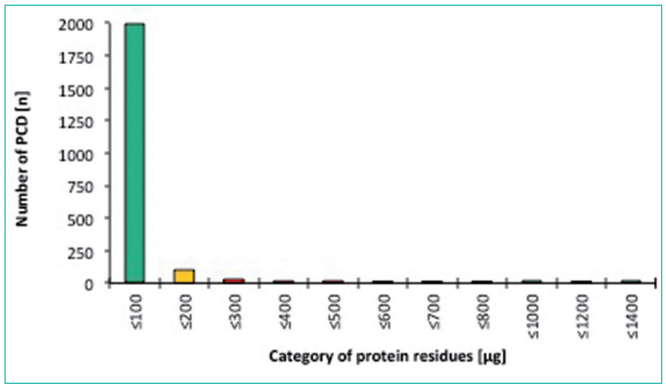

Fig. 4: Distribution of the frequency [n] of test pieces (PCD) classified into the respective category of residual protein content [µg test piece^{-1}] (n = 2171).

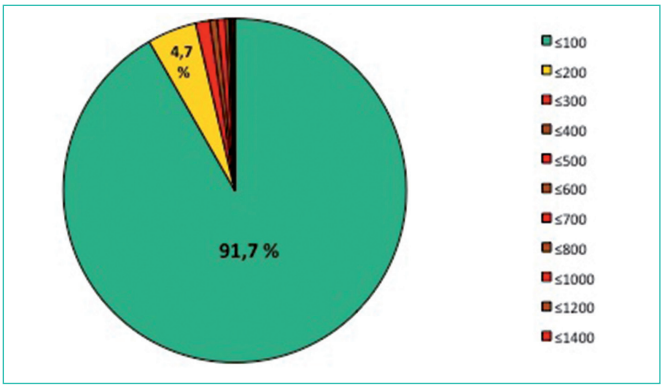

Fig. 5: Distribution of the relative frequency [%] of test pieces classified into the respective category of residual protein content [µg test piece^{-1}] (n = 2171 [≈100 %]).

Discussion

Established acceptance criteria

At the time the guideline was published (2011) there was no significant number of data available on the residual protein content of test pieces that underwent automated cleaning processes in practice to allow for the definition of acceptance criteria or minimum requirements, respectively. The requirements were therefore defined with reference to the publication AAMI TIR30:2003 [8] that claimed a protein-surface-value of < 6.4 µg protein cm^{-2}. This value was ascertained by Alfa *et al.* by quantification of residual proteins in biopsy channels of manually reprocessed endoscopes after practical usage [10, 11].

Recent data from 2014 on the achievable residual protein content in biopsy channels after manual cleaning and subsequent pump-assisted flushing using enzymatic detergents led to a reduction of the recommended protein-surface-value to < 2 µg protein cm^{-2} [13]. Detailed investigations of the successive protein reduction by the respective reprocessing steps: i) immediate pre-cleaning, ii) brush cleaning, iii) automated cleaning in an EWD and iv) entire reprocessing in an EWD were conducted and published by Pineau and De Philippe in 2013 [14]. These investigations showed that after brush cleaning the residual protein content averaged 0.9 µg cm^{-2}, while residues amounting to 6.7 µg cm^{-2} after automated cleaning in an EWD. The results were interpreted as artefacts due to matrix effects caused by residues of cleaners that interfered with applied protein quantification methods.

Test pieces as process controls

Published data on the residual protein content in biopsy channels of real instruments after manual or automated reprocessing [10, 11, 13, 14] cannot be referred to for the definition of acceptance criteria for the test piece model. The reasons are several systematic differences:

– Test pieces are used within PQ testing as process controls for the evaluation of the minimum requirement for cleaning efficacy. A general requirement for test pieces is good reproducibility. The artificial soiling of Annex 8-test pieces is done with reactivated (preferably pooled) sheep blood. In contrast to that, real instruments show a high diversity of different soil types and soiling levels, which differ depending on the site of operation and the respective patient.

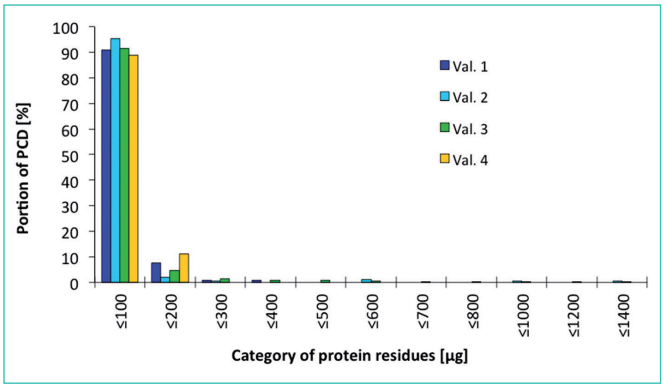

Fig. 6: Distribution of the relative frequency [%] of the test pieces (PCD) classified into the respective category of residual protein content [µg test piece^{-1}] displayed for the individual validation laboratory [Val.] (n = 2171 [≈100 %]).

– Test pieces are required to allow for the quantification of the cleaning efficacy over a wide range. The soiling is therefore done with a high amount of test soil, which is well above average soiling levels detected in the biopsy channel of real instruments. In the DEGEA study [7], 60 positive control test pieces were analyzed that showed an average amount of soil exceeding 67,000 µg protein/test piece.

– For the preparation of test pieces new PTFE tubings are used. These feature a smooth surface. The surface topology of tubing materials supplied by different manufacturers differs only insignificantly on a micro-scale. In contrast to that, the channel surfaces of real instruments show an undefined surface topology. This is especially true for biopsy channels that show a highly diverse surface roughness caused by mechanical wear if accessories (biopsy forceps, snares, cleaning brushes, etc.) are repetitively inserted.

– Alfa *et al.* as well as Pineau and De Philippe eluted residues using water as eluent. In contrast, an alkaline sodium dodecyl sulphate solution (1% SDS, pH = 11) was established as eluent with the highest elution efficacy for protein residues a long time ago. The latter eluent is referred to by many other guidelines and methods for protein quantification [12, 15, 16, 17].

Results of the field investigation

The presented results of the field investigation were obtained by testing the cleaning efficacy of current EWD processes applying Annex 8-test pieces. The testing was performed within the scope of the initial or repeated PQ testing in endoscopic facilities. The participating validation laboratories offer their validation service to customers all over the country. Therefore it is assumed that provided results are representative for commonly applied practice relevant reprocessing procedures.

For the testing of 888 EWD processes, 2298 test pieces were applied. The majority of the test pieces were 1 – 6 days old, the cumulative relative share of test pieces with an age of ≥ 7 days was 5.6%. According to Annex 8 the prepared test pieces are intended for immediate application. For the application after storage the manufacturer has to give a proof or assure the equivalence of results, respectively.

Qualitative evaluation

Of the 2298 test pieces 127 showed residues, i.e. coagula, when inspected visually. Like in the DEGEA study [7], a principal correlation between visually detectable residues and the residual protein content was observed. However, in this field investigation, 8.3% (n = 181) of the visually clean test pieces (n = 2171) exhibited a residual protein content of > 100 µg protein/test piece. The observed maximum was 1365 µg/test piece. These test pieces gave false-negative results with regard to the visual inspection. The results implicate that the assessment of cleaning efficacy cannot be done by visual inspection solely, but must be done by a subsequent evaluation of both acceptance criteria. Protein residues in visually clean test pieces can in principal occur, if the haemoglobin of the blood soil is washed out quantitatively during the cleaning and intermediate rinsing step. Remaining fibrin soilings have a whitish appearance and cannot be detected easily through the opaque walls of the test pieces. In principal, protein residues can also occur due to a carry-over of soil and/or residues of enzymatic cleaners and their laminary dispersion on inner surfaces of test pieces. The data set of this field investigation doesn't contain any information about the usage of enzymatic cleaners; therefore their application can not be excluded. However, in the DEGEA study the majority of non-soiled test pieces (negative controls) showed small protein amounts below the limit of quantification. The soil-carrying properties of cleaners were judged to be good and the influence of contained enzymes was assumed to be negligible.

In contrast, within the category of test pieces containing 1 – 2 coagula (n = 18) 56% of the test pieces exhibited a residual protein content of 0 µg/test piece. Within the category of test pieces with 3 – 10 coagula (n = 19), the portion of test pieces with an residual protein content of 0 µg/test piece was 16% and within the category of test pieces with > 10 coagula (n = 90) it was 12%. The reason for the non-detectability of protein residues that were visually detectable is probably caused by an inefficient elution due to previous denaturing effects (temperature in combination with chemistry), that resulted in a diminished solubility of residual proteins in SDS solution.

Quantitative evaluation

74.9% (n = 1626) of the visually clean test pieces did not contain quantifiable residual proteins, results for these test pieces were reported as 0 µg. Methods for protein quantification should be characterized by the applying laboratory, just as other analytical methods. Characterization can be done using DIN 32645 [18] which allows the determination of the limit of detection, the "Erfassungsgrenze", and the limit of quantification (LOQ). The latter gives the threshold of the minimum amount of the analyte (here the protein amount) that can be quantified within the chosen range of uncertainty. The data set of this field investigation did not contain any information about the limit of detection and the limit of quantification. Thus, results reported as 0 µg could not be precisely distinguished to be below the limit of quantification or detection. In the DEGEA study [7], the limit of quantification of the used OPA method was estimated to be 10 µg/ml, consequently the lowest quantifiable protein amount was 50 µg/test piece within the chosen uncertainty. For a consistent analysis, results with 0 µg/test piece and results with × ≤ 100 µg/test piece were combined. The portion of visually clean test pieces which fell into that category of residual protein content was 91.7%. The high percentage of test pieces with low residual protein content substantiates that the tested EWD processes feature a high cleaning efficacy. As little as 0.3% of the visually clean test pieces showed a residual protein content of > 800 µg and thus exceeded the actual guide value. The actual limit value of > 1600 µg protein was not exceeded by any of the test pieces.

The present data of this field investigation implicate that the acceptance criteria for the quantitative evaluation of the residual protein content have to be adjusted. The members of the guideline group, consisting of representatives of DGKH, DEGEA, DGSV, DGVS, AKI and manufacturers of endoscopes and EWD, have jointly decided to lower the acceptance criteria on the basis of the existing results.

Data consistency

The data of the four independent validation laboratories showed a good agreement of the results. The differences of the relative frequency of test pieces within the residual protein category × ≤ 100 µg/test piece amounted to 6.4%, for the category 100 < × ≤ 200 µg/test piece to 9.0% (referred to the overall number). These differences were assumed to be very low and demonstrate: i) that the differing efficacies of cleaning processes in the field were sufficiently equilibrated by the high number of results, ii) that no systematic differences in preparation, application and evaluation of the test piece model were existent between the different validation laboratories, consequently a good comparability of results was yielded, iii) that the test piece model provides a good reproducibility over the 6 month period of data collection.

Actualization of acceptance criteria

On the basis of the present results and in due consideration of the actual state-of-the-art of automated endoscope reprocess-

ing procedures, the following acceptance criteria for the evaluation of the cleaning efficacy, applying Annex 8-test pieces, are proposed:

1. Acceptance criterion:

– visually clean test pieces

2. Acceptance criterion:

– Guide value ≤ 100 µg protein/test piece
– Warning level > 100, ≤ 200 µg protein/test piece
– Limit value > 200 µg protein/test piece

To comply with the minimum requirements regarding cleaning efficacy, the first acceptance criterion of visual cleanliness as well as the guide value with respect to the residual protein content have to be met.

The reduction of the guide value for the quantitative evaluation to ≤ 100 µg protein/test piece leads to an equivalence with the warning value for tolerable residual proteins on reprocessed medical devices according to the joint recommendation of KRINKO/BfArM [19]. It is pointed out that the test pieces according to Annex 8 are process controls, which as a surrogate exhibit properties that refer to the characteristics of relevant medical devices.

The publication of the actualized acceptance criteria is issued in parallel in *Central Service* in a separate information of the guideline group and will be implemented in the actual revision of the guideline. ■

Acknowledgements

The author thanks the participating validation laboratories for the provision of result data and all members of the guideline group as well as W. Michels (Warburg) for detailed revision, vivid discussions and support.

References please see p. 218