Round robin tests conducted by the working group DaVinci («AG DaVinci») to establish a method for testing the cleaning of MIS robotic instruments

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In order to quantify the cleaning efficacy for MIS robotic instruments after reprocessing, the working group DaVinci established two methods to elute residual protein soil from robotic instruments and to subsequently quantify it [1]. While developing the methods, round robin tests were conducted and recovery rates were quantified using a model protein (bovine serum albumin, BSA, Fraction V) as well as reactivated sheep blood as test soil. The amount of test soil applied was between 200 – 500 µg protein, referred to bovine serum albumin (BSA), and thus within a critical range to the acceptance level for reprocessed instruments. The recovery rates determined using a clinically relevant test soil of reactivated sheep blood were on average 96 % (non-destructive elution method), or 83 % (destructive elution method). For protein quantification, the modified ortho-phthaldialdehyde (OPA) method [2, 3], as well as the bicinchoninic acid (BCA) method [4] were used. Both methods provided comparable results. However, for both methods influences were observed that were attributed to interfering substances from the instruments. Methods to remove these interfering compounds from the eluates could not be established yet. Experimental series conducted by single laboratories showed that recovery rates were in good match for varying amounts of test soil ranging from 100 – 1000 µg per instrument. Applying the described methods recovery rates showed a good correlation between the participating laboratories, taking into account the complexity of the medical devices analyzed.

The composition of the working group DaVinci is represented by the authors of this publication. Representatives from Intuitive Surgical Inc., Sunnyvale, CA, USA participated as guests and supported the group by providing instruments, among other things.

KEY WORDS
- robotic instruments
- DaVinci
- cleaning
- residual protein
- test soil
- modified OPA method
- BCA method
- recovery rate

Introduction

Robotic systems for minimally invasive surgery (MIS) manufactured by Intuitive Surgical Inc. (Sunnyvale, CA, USA) [5] are being increasingly deployed in German hospitals (70 hospitals by February 2014). During surgery using the systems DaVinci®, DaVinci S®, and DaVinci Si®, three surgical instruments and a high resolution 3-D camera system are used. The surgical instruments (EndoWrist instruments) are cleared by the manufacturer for different numbers of procedures, depending on the respective type, design and geometry of the instrument. Reprocessing consists of a manual pre-treatment and subsequent automatic cleaning and thermal disinfection in washer-disinfectors (WD) with specific loading carriers [6, 7] as well as a final sterilization step. During process validation [8, 9] the reprocessing procedures undergo performance qualification (PQ). Moreover, periodic tests using instruments contaminated during actual use are required [10, 11].
Recently, methods were established and published by the working group DaVinci [1] that allow the determination of residual soil using protein as the main parameter. Application of these methods affords, for the first time, a consistent evaluation of the cleaning efficacy by different test laboratories. In addition to the described methods, a recommendation for acceptance criteria is given, based on a surface-related maximum residual protein content of currently 3 µg/cm² [14, 15]. The application of these acceptance values is in accordance with actually published guidelines [10, 16, 17]. The published test methods were developed by the members of the working group DaVinci in collaboration with the manufacturer of the instruments, and were evaluated and proved in round robin tests. Maryland Bipolar Forceps (MBF) served as model instruments for the investigations. Relevant results collected in round robin tests are depicted as follows. Recovery rates were determined for the non-destructive elution method (Type I testing), the destructive elution method (Type II testing), using various test soils (BSA and reactivated sheep blood) as well as various amounts of test soil.

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Material and methods

Solutions and Equipment

Solutions, equipment and consumables needed for the elution of soils are listed in detail in [1]. Regarding protein quantification methods reference is made to the chapter «Protein quantification methods». The following materials were used for the soiling of MBF instruments:

- Sheep blood heparinized with 10 IE heparin ml^{-1}. «Pooled» blood, i. e. a mixture of blood from several animals was used, ordered from Acila GmbH.
- Protamine sulphate, applied to a final concentration of 15 IE ml^{-1} blood. This product was obtained from the blood supplier.
- 0.9 % physiological sodium chloride solution (NaCl solution), sterile
- Bovine serum albumin (BSA), Fraction V, purity ≥ 98 % (Carl Roth, order no.: T844.2)
- PTFE capillary tubing: tube with external ∅: 1.06 mm and interior ∅: 0.60 ± 0,07 mm (Reichelt Chemietechnik, Thomafluid PTFE Chemieschlauch, order no.: 25262) including an adaptor to connect to a disposable 10 ml syringe

Methods

Design of the round robin tests

Prior to a round robin test with the seven laboratories, all instruments (Maryland Bipolar Forceps, MBF) were subjected to a uniform pre-treatment and conditioning by laboratory B (Chapter «Pre-treatment of instruments»). Afterwards, laboratory C conducted the soiling of the instruments (Chapter «Test soil»), subsequently the instruments were shipped to each laboratory by express mail. During shipment the instruments were cooled and the conditions monitored by thermo-loggers. Each laboratory received 3 soiled MBF instruments as well as a non-soiled instrument as a negative control. In the laboratories elution of the soil was carried out according to a uniform experimental protocol [1]. For the quantification of the protein content of the eluates the laboratories were free to chose between the modified OPA or the BCA method.

Pre-treatment of instruments

The instruments used for a round robin test were subjected to a uniform pre-treatment («baselining») procedure, to ensure a reproducible elimination/removal of undefined protein soil and/or a reduction of interfering substances. At first the instruments were eluted with 1 % SDS solution (pH = 11), to quantify the initial protein or interfering substance content before reprocessing. Subsequently 3 repetitive reprocessing cycles, consisting of cleaning, disinfection and sterilization were carried out. Cleaning and disinfection was carried out in a WD (Medisafe, type: Niagara SI PCF) with a specific loading carrier for robotic instruments. The particular process steps were: pre-cleaning (2 min 45 s; 32 °C; applying ultrasound), cleaning (9 min 15 s; 43 °C; 0.75 % 3E-Zyme (Medisafe); applying ultrasound), rinsing (3 min; 50 °C) and thermal disinfection (3 min; 91 °C). Sterilization was simulated by treatment in a steam sterilizer (MMM, Type: Selectomat HP) (5 min; 134 °C; drying step 20 min) without sterile packaging. The effect of these 3 repetitive reprocessing cycles was checked by two subsequent elution steps of all instruments using 1 % SDS solution (pH = 11), and the quantification of protein residues using the modified OPA method. The SDS solution was removed from the instruments applying a final reprocessing cycle in the WD, without the addition of detergents.

Test soil

The test soil consisted either of 1 mg ml^{-1} BSA solution (Fraction V), or «pooled» heparinized sheep blood. The blood was reactivated by addition of the respective amount of protamine sulphate and gentle but thorough mixing. The test soil was applied immediately after preparation. To quantify the protein content, the BSA solution was diluted by the factor of 1 : 10, the blood soil by the factor of 1 : 1000 in 1 % SDS solution (pH = 11). The protein content, quantified as equivalent referred to BSA, Fraction V, was determined using the modified OPA method.

Soiling of the distal working end

The soil was applied onto the opened jaws, the Bowden cables and the pulleys. The soil was spread by moving the distal working end and the jaws several times in each direction. Soiling was carried out with 100 µl BSA solution (1 mg ml^{-1}) or with 2 µl of reactivated sheep blood, Fig. 1. To quantify the protein content of the applied blood, 10 measurements were carried out, therefore the blood (2 µl) was diluted in 998 µl 1 % SDS solution (pH = 11), and then protein content was quantified using the modified OPA method.

Soiling of the shaft interior

The soiling of the shaft interior of the MBF instruments was carried out at the distal part, where the Bowden cables pass through the septum plate. For soiling a PTFE capillary (external ∅: 1.06 mm) was introduced into the flush tube of the instrument (flush port 1) and pushed down to the distal end. The test soil consisting of 100 µl BSA solution (1 mg ml^{-1}) was injected into the capillary using a microliter pipette. The complete amount of soil was expelled and deposited by injection of air. For soiling with small volumes of blood the so-called «muzzle-loader method» was developed. 2 µl of the reactivated blood soil were pipetted into the lumen at one end of the capillary tube (Fig. 2A). The filled capillary was photographed (Fig. 2B) and this soil-loaded end introduced into the flush tube (flush port 1) of the MBF instrument (Fig. 2C). The blood soil was blown out at the septum area by injection of 10 ml air (Fig. 2D). Subsequently the 15 – 20 mm long portion of the capillary that had previously been filled with the blood soil was cut off with a scalpel and eluted with 1 ml 1 % SDS solution (pH = 11, 30 min incubation, periodic vortexing). Eluted blood residues were quantified using the modified OPA method. The analysis of the photograph taken of the loaded capillary served for the subsequent calculation of blood volume loaded into the capillary. By subtracting the amount of eluted residues from the capillary after soiling, the amount of applied blood was exactly determined.

In order to quantify the blood volume loaded into the capillary tube as exactly

Fig. 1: Soiling of the distal working end by applying 2 µl of reactivated sheep blood.

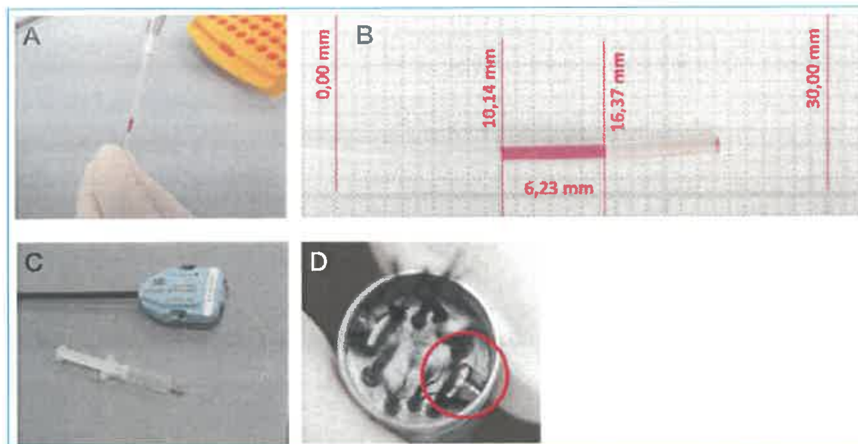

Fig. 2: Soiling of the shaft interior. A: Loading of the PTFE capillary tube with approximately 2 µl blood using a microliter pipette. B: Digital image interpretation of the loaded PTFE capillary tube. C: The loaded capillary tube is inserted into flush port 1, pushed down to the distal end of the instrument and the soil was expelled by injection of air. D: Dismounted distal working end with blood soil visible in the cylindrical part close to the septum plate.

as possible, the PTFE capillary tube was calibrated before the first use. For this, 11 sections of the PTFE capillary tube were cut and the weight measured using an analytical balance with a resolution of 100 µg. The sections were filled with blood (1.1 – 2.4 µl), photographed and the length of the filled capillary volume was determined (Fig. 2B) using digital image interpretation. The amount of blood introduced was determined gravimetrically. Subsequently the blood was eluted with 1 ml 1 % SDS solution (pH = 11), and the protein content was quantified using the OPA method. The mathematical correlation between the length of the filled capillary volume and the contained amount of blood related to protein content was: $y = 2.92 \times$ (n = 9). The correlation relative to the weight of injected blood was: $y = 3.08 \times$ (n = 11). To calibrate the capillary, a consensus equation $y = 3.00 \times$ was used. Based on these data, the interior diameter of the used lot of PTFE capillary tube was calculated as 0.67 mm.

Protein quantification methods

The modified OPA method [2, 3] and BCA methods [4] using photometers for quantitative measurements were applied. Chemicals used are listed in EN ISO 15883-1 Appendix C [4]. Alternatively commercially available test kits were used (e. g. INTERCHIM BC Assay Protein Quantitation Kit (order no.: UP40840A); QuantiPro BCA Assay Kit (Sigma-Aldrich, order no.: QP-BCA); Miele Test Kit [12, 13] (Merk KGaA) in combination with the reflectometer RQ-flex® plus 10 Reflectoquant® (Merck, order no.: 1169550001).

Results

Recovery of various amounts of BSA soil from the shaft interior using the non-destructive method (Type I) – preliminary tests

An experimental test series was conducted by laboratory B to determine the recovery rate (RR) for an artificial test soil of BSA, Fraction V, after elution from the shaft interior of the robotic instruments. Moreover the possible influence of varying amounts of soil on the recovery rate was investigated. Groups of three MBF instruments were soiled with 100, 250, 500 and 1000 µg BSA, respectively, within the shaft element. To apply the soil, four different BSA solutions with concentrations of 1000, 2500, 5000 and 10000 µg ml^{-1} were prepared. Each instrument was soiled with 100 µl of the respective BSA solution by introduction of a PTFE capillary tube through the flush tube of the instrument and by depositing the soil at the distal septum area. The instruments were stored for about 1 hour at room temperature. After this, elution of the shaft element was carried out as described for Type I testing [1], but using 7 ml SDS solution (1 %, pH = 11). Protein quantification was done using the OPA method. For the non-soiled control instruments, a protein content of 48.8 ± 5.60 µg was determined (n = 8). These readings were caused by interfering substances causing false-positive results, see Fig. 3A. The correction of the protein content of eluates from soiled instruments by the value obtained from control instruments, yielded recovery rates (RR) of 69.1 – 79.3 % with respect to the different amounts of applied soil (Fig. 3B).

Recovery of BSA soil from the distal working end and from the shaft interior using the non-destructive method (Type I)

The laboratories B, C, F and G took part in a round robin test to assess the efficiency of elution and quantification of artificial test soils from instruments. For the test, 12 MBF instruments were soiled with BSA, another 4 instruments were not soiled and served as control instruments (negative control). The soiling consisted of 100 µg BSA (100 µl BSA solution, concentration 1 mg ml^{-1}) applied to the distal working end and another 100 µg BSA injected into the shaft element deposited at the distal septum area. After drying of the soil, the instruments were packed cooled on dry ice and shipped to the participating laboratories. For the elution of the instruments, the published Type I method [1] was applied with the modification that the elution of the distal working end was done with 4 ml SDS solution (pH = 11) and the elution of the shaft interior with another 7 ml fresh SDS solution. Each eluate sample was analysed separately. For the protein quantification, the modified OPA method (laboratory B, C, G) and the BCA method (laboratory F, G) were applied. Laboratory G carried out measurements using both methods.

For non-soiled control instruments, varying protein contents up to 51 µg were measured by the participants. The data are depicted in Fig. 4A. Measured protein contents are false-positive readings, as a protein contamination was ruled out because of the pre-treatment procedure (baselining). These readings were presumably caused by interfering substances whose origin and chemical nature have not been clarified yet. Because of the strongly varying false-positive values for the control instruments from different laboratories, no correction of measured protein contents of soiled instruments via subtraction of the readings for control instruments was carried out.

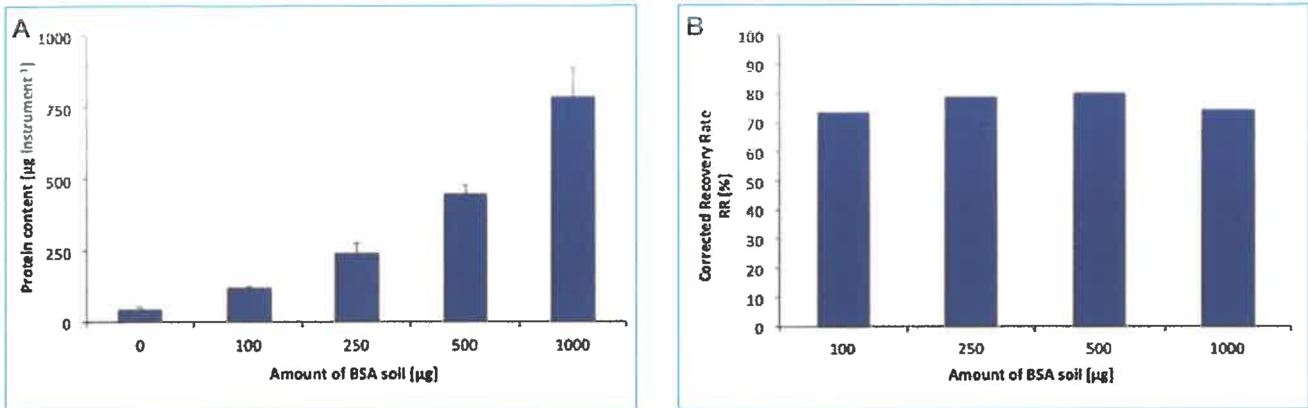

Fig. 3: Elution and quantification of various amounts of BSA test soil (100 – 1000 µg BSA per instrument) from the shaft interior of MBF instruments and calculation of recovery rates. A: Residual protein content measured from eluted soiled instruments (n = 3) and non-soiled control instruments (n = 8). (error bars: standard deviation). B: Recovery rates (RR, %, n = 3) after subtraction of the average measured protein content of the control instruments (n = 8).

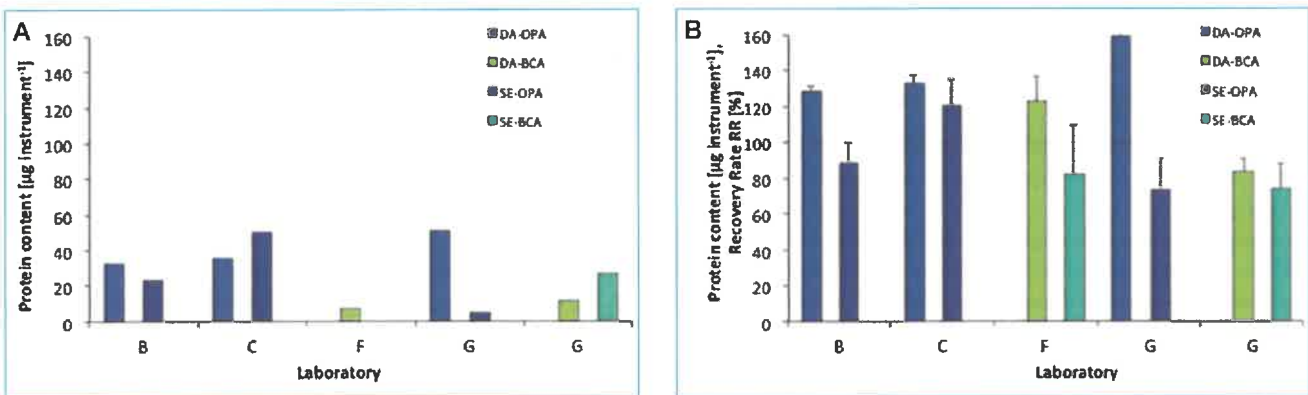

Fig. 4: Recovery rates for a test soil of 100 µg BSA both on the distal working end and in the shaft element of MBF instruments using the non-destructive Type I-elution. A: Varying false-positive readings were obtained for the non-contaminated control instruments (negative controls, n = 1 instrument per laboratory). B: Recovery rates for soiled instruments (n = 3 instruments per laboratory). (DA: distal working end, SE: shaft element, OPA/BCA: applied protein quantification method, error bars: standard deviation).

The recovery rates (RR) for a soiling with 100 µg BSA on the distal working end and within the shaft element are depicted in Fig. 4B. The recovery rates for the distal working end (without correction for negative controls) ranged between 77.3 – 166 %, the arithmetic mean was 125 ± 26.7 % (n = 15). For the shaft element the recovery rates ranged between 49.0 – 138 %, the arithmetic mean was 87.7 ± 25.9 % (n = 15). Subsequently, the measured protein content of the distal working end and of the shaft element were added. The recovery rates for the 15 measured eluates were calculated, these ranged between 69.3 – 136 %, the arithmetic mean was 106 ± 19.6 %.

Recovery of blood soil from the distal working end and from the shaft interior, non-destructive method (Type I)

The laboratories A, B, C, D, E and F participated in a round robin test with the aim to assess recovery rates for a soil consisting of reactivated sheep blood. Both, distal working end and shaft interior were soiled with 2 µl sheep blood. In order to increase the sensitivity of the method, the volume of eluate utilized was reduced in comparison to previous round robin tests. A total of 6 ml SDS solution (pH = 11) was used to elute successively the distal working end and then the shaft element. The procedure used for this round robin test was in accordance with the published method of the working group DaVinci [1]. To quantify the recovered test soil the modified OPA method (laboratory A, B, C, D), as well as the BCA method (laboratory A, B, C, E, F) were used. Calculated recovery rates are shown in Fig. 5.

In the round robin test the six participating laboratories tested three soiled MBF instruments each. In addition, each laboratory tested a non-soiled control instrument. However, data from negative controls were not used for a correction of measured protein contents of soiled instruments and the determination of recovery rates. As several laboratories used both protein quantification methods to analyse the eluates, a total of 30 individual results were collected. The

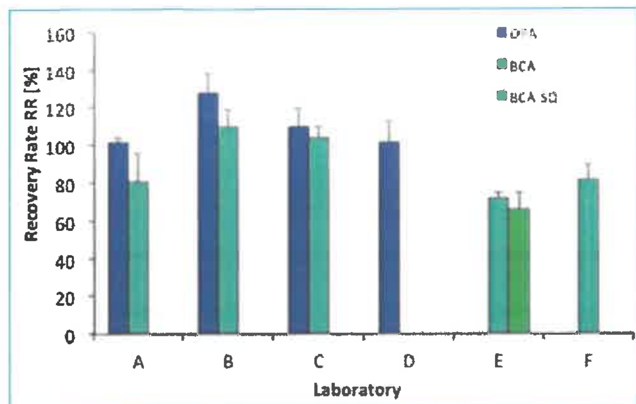

Fig. 5: Recovery rates for a test soil of 2 µl sheep blood each on the distal working end and in the shaft element of MBF instruments after a non-destructive Type I elution (n = 3 instruments per laboratory; error bars: standard deviation; OPA and BCA: applied protein quantification method, BCA-SQ: BCA method with simplified reflectometric measurement).

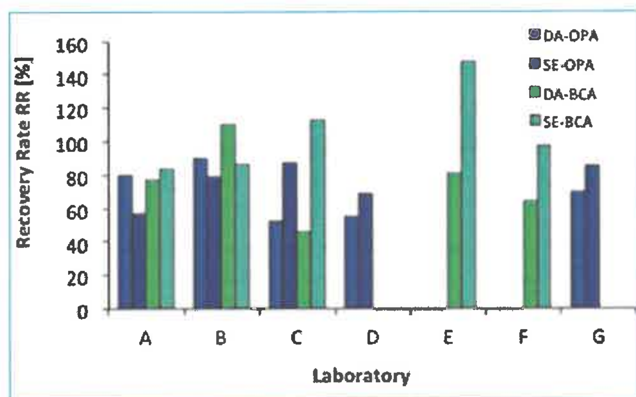

Fig. 6: Recovery rates for a test soil of 2 µl sheep blood on the distal working end and of 2 × 2 µl in the shaft element of MBF instruments using the destructive Type II elution (n = 2 instruments per laboratory; DA: distal working end, SE: shaft element, OPA/BCA: applied protein quantification method).

recovery rates ranged between 55.9 – 139 % and the arithmetic mean was 95.5 ± 20.5 %.

Recovery of blood soil from the distal working end and from the shaft element using the destructive method (Type II)

A round robin test conducted by the laboratories A, B, C, D, E, F and G aimed at assessing the recovery rates for the destructive elution method (Type II). The MBF instruments were soiled with 2 µl reactivated sheep blood each on the distal working end, in the shaft element near the distal septum plate, and also approx. 15 cm above the distal septum plate. This soiling positioned towards the middle of the shaft element was likewise deposited with the already described «muzzle-loader method». Therefore the PTFE capillary tube was introduced as described before. The PTFE capillary and the flush tube were then retracted in parallel by 15 cm. To retract the flush tube the blue housing cover of the instrument was pryed off and the flush port 1 was loosened out of its location. Each of the seven participating laboratories tested 2 soiled instruments. The applied elution procedure in this round robin test was in accordance with the published method in [1] and delivered one result for the distal working end and one for the shaft element for each instrument. As several laboratories applied both protein quantification methods to analyse the obtained eluates, in total 40 individual results were collected. The calculated recovery rates are summarized in Fig. 6.

The recovery rates for the elution of the distal working end ranged between 43.9 – 114 %, the arithmetic mean was 72.6 ± 27.7 % (n = 20). For the shaft element, the recovery rates were 56.4 – 154 % the arithmetic mean was 90.7 ± 25.4 % (n = 20). Soiling of the shaft interior could result in a deposition of the soil on the distal septum plate as well as on the nearby Bowden cables. When dismantling the instruments the soil might be localized on the distal working part or parts attributed to the shaft element. Because of this uncertain location and attribution of the test soil the measured protein content of distal working end and shaft of each instrument was added and overall recovery rates were calculated, results are shown in Fig. 7. Overall recovery rates ranged between 60.4 – 128 %, the arithmetic mean was 83.1 ± 16.6 %.

Discussion

The objective of the working group DaVinci was to establish, specify and describe a reproducible method to quantify the residual protein content from robotic instruments that had been contaminated by actual use and then reprocessed. Residual protein contents of significantly < 500 µg are expected on these instruments; these must be reliably detected and quantified. The robotic instruments are cleared for a specific number of uses/reprocessing cycles by the manufacturer. For this reason, two different elution methods were established: the non-destructive Type I elution for instruments which have not yet reached their maximum number of usages/reprocessing cycles, as well as the destructive Type II elution to test instruments after the maximum number of usages/reprocessing cycles. In particular the Type II elution at the «end of the instrument's life» allows for a determination of residual proteins that might have accumulated during the entire number of usages.

The working group conducted in total five round robin tests using 28 MBF instruments. The results of three round robin tests are reported in this article along with test results from individual laboratories. Up to now, the presence of yet unidentified substances is a challenge as these interfere with the two established protein quantification methods, i. e. OPA and BCA, during quantification of residual protein contents of robotic instruments.

These substances could not be removed quantitatively from instruments that had been employed during 5 round robin tests and that had been subjected to the respective «baselining» pre-treatment consisting of repetitive cleaning-disinfection processes. Interfering substances are present in the eluates from the distal working end as well as from the shaft element. Tests conducted by laboratory C to remove these interfering substances, using organic solvents (chloroform, cyclohexane, ethyl acetate, xylol) for extraction procedures, as well as the use of protein purification columns, had no effect. Eluates free of protein and treated

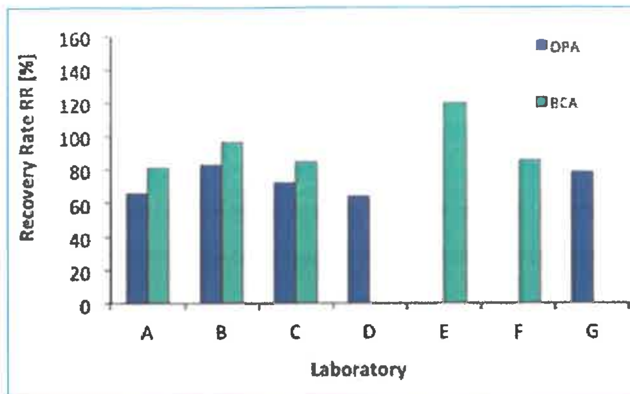

Fig. 7: Recovery rates for the overall protein content distal working end + shaft element (n = 2 instruments per laboratory; OPA/BCA: applied protein quantification method).

by solvent extraction gave wrong protein readings when spiked with defined amounts of BSA (50 µg ml^{-1}). Deviations from the actual protein content were up to approx. 50 µg each for the eluate of the distal working end and the shaft element, irrespective of applying the BCA or the OPA method. In parallel investigations of laboratory C showed that the addition of an instrument conditioning oil (1 µg ml^{-1}, e. g. Sterilit, from Braun Aesculap, Tuttlingen) showed no interfering effects on the BCA or OPA method. The influence of interfering substances varied strongly depending on the particular instrument eluted as showcased in Fig. 4A. The results for all negative control instruments analysed by the participating laboratories are given in table 1. Table 2 summarizes the measured false-positive protein readings that were gained from the instruments during the «baselining» pre-treatment procedure using the modified OPA method.

As false-positive results were not a constant systematic error, it was not possible to make corrections. This circumstance was implemented in the way that measured protein contents of instruments were not corrected for values of unsoiled instruments. This leads to an overestimation of actual protein contents. However, as the recovery rate for residual soil is always below 100 %, it is assumed that both influences cancel each other out, at least partially. Indication for an inhibition («false-negative») were not obtained.

The test series conducted by laboratory B demonstrated that the elution method is suitable to quantify varying amounts of protein in the shaft element. By varying the amount of soil from 100 to 1000 µg BSA per instrument, recovery rates between 69.1 – 79.3 % were obtained. Consequently varying relevant amounts of soil can be recorded with comparable efficiency.

The results of the round robin tests showed that regarding the method of protein quantification both the BCA and the modified OPA method could be used. Neither method showed systematic advantages with respect to the influence of varying amounts of interfering substances present.

By using BSA as a test soil, slightly higher recovery rates were measured compared to reactivated sheep blood. This result was expected, as BSA is fairly soluble in water, whereas coagulated sheep blood is a soil of highly complex composition, consisting of countless proteins and a fibrin network of high molecular weight that presents a high challenge to cleaning processes, as well as for elution methods. For the non-destructive Type I elution recovery rates of 95.5 % were reported, whereas the recovery rates for the destructive Type II elution were 83.1 %, see table 3. Both values are in a similar range. Differences and the scatter of results can be attributed to the analysis in the various laboratories, the application of various protein quantification methods and the inconstant and non-quantifiable influence of interfering substances. If it is assumed that interfering substances make a false-positive contribution of 10 %, the recovery rates would be approx. 85 %, or approx. 73 %. This would be consistent with the prevailing recovery rates for other geometrically complex test objects such as the test pieces of appendix 8 [3] of the guideline for the validation of cleaning and disinfection processes for thermo-labile endoscopes by washer-disinfectors.

All laboratories yielded sufficiently high recovery rates for test soils in the range of less than 500 µg protein, that was assumed to be relevant for the evaluation of reprocessing procedures. Hence two methods were established that allow for the first time a comparable evaluation of reprocessing procedures at different testing laboratories.

Table 1: Summary of false-positive protein contents on unsoiled negative control instruments measured by participating laboratories using respective quantification methods. Negative control instruments were eluted and analysed similar to soiled instruments (DA: distal working end, SE: shaft element, LOD: limit of detection).

No. of round robin test, resp. figure	Elution method	Quantification method	Laboratory	False-positive protein content (DA/SE), [µg instrument^{-1}]
No. 1, Fig. 4	Type I	OPA	B; C; G	(32.5/23.1); (35.5/50.4); (50.8/4.9)
		BCA	F; G	(7.1/<LOD); (11.5/27.1)
No. 2, Fig. 5	Type I	OPA	A; B; C; D	14.3; 44.1; 28.8; <LOD
		BCA	A; B; C; E; F	<LOD; <LOD; 34.9; <LOD; <LOD
No. 3, Fig. 6/7	Type II	*No negative controls used*		

The amount of the applied reactivated sheep blood was significantly above the acceptance criterion as smaller amounts cannot be precisely deposited. Moreover smaller amounts show a reduced crosslinking during coagulation so that the blood soil would be easier accessible and easier to elute with SDS solution.

The published methods for testing the cleaning [1] include a recommendation for acceptance criteria. The first acceptance criterion is the absence of visually detectable soil residues. The second acceptance criterion is a maximum residual protein content based on the surface area with a value of 3 µg cm^{-2} [14]. The value of this surface-related protein content is based on the evaluation of results from performance tests on real-life instruments [15] and represents the state of the art expected to be met. The surface-related approach is also implemented by the guideline of DGKH, DGSV, AKI and VAH for the validation of manual cleaning and manual chemical disinfection procedures for medical devices [16]. Moreover it is implemented by the revised acceptance criteria of the guideline of DGKH, DGSV, and AKI for the validation and routine monitoring of automatic cleaning and thermal disinfection processes for medical devices [17]. Also the standard committee ISO 198 WG13, who is currently revising ISO/TS 15883-5, is likewise considering setting up acceptance criteria on the basis of the surface-related approach, even for further parameters such as TOC (total organic carbon) content. Due to the large variety of medical devices to be evaluated, the residual protein to surface ratio is a trendsetting and meaningful dimension for the assessment of residual contaminations.

Table 2: Summary of false-positive protein contents of instruments assessed during the «baselining» pre-treatment of the instruments.

Number of automatic reprocessing cycles	Number of instruments	False-positive protein content [µg $instrument^{-1}$] incl. standard deviation
0	20	117 ± 24.6
3	20	54.6 ± 11.3
6	8	48.8 ± 5.6
10	20	29.2 ± 6.5

Table 3: Summary of recovery rates determined in round robin tests using different test soils and different elution methods.

Elution method	Test soil	Number of instruments	Number of measurements	RR ± SD
	BSA: 2 × 100 µg	12	15	106 ± 19.6 %
Type I	Blood: 2 × 2 µl	18	30	95.5 ± 20.5 %
Type II	Blood: 3 × 2 µl	14	20	83.1 ± 16.6 %

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