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Revision 01 **Project Number: 13616**

Examination Report
Medizin-Mechanik-Nord GmbH

Automated Cleaning Process
Using the Radionuclide Method and Quantitative Protein Detection

(Method MD 1.1 and MD 1.2)

Global – Limiter with silicone handle
Self – Retaining Screwdriver

Medizin-Mechanik-Nord GmbH
Russeer Weg 54a
24111 Kiel

Sponsor Address

28-April-2016	4179-051201	02-May-2016
Order Date	Order Number	Delivery Date

20-June-2016 - 30-June-2016	-
Examination Period	Remarks

06-July-2016
Date of Report

Signatures

Responsible for the Method
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Quality Management:
Dr. Ludger Schnieder

Revision	Date	Occasion
01	04-July-2016	Initial compilation of the report

1 Objective of the Examination

The objective of this project was to examine the cleaning behavior of the samples listed in Table 4 in an automated cleaning process.

Acceptance Criteria

A selection of acceptance criteria for evaluating the cleaning success of reusable medical devices found in national and international standards, guidelines and other documents is listed below.

No.	Criterion	Acceptance level	References
1	There shall be **no soil visible** on the sample at the end of the cleaning process.	N/A	EN ISO 15883-1: 2006 RKI guideline: 2012 AAMI TIR 30: 2011 Guideline DGKH, DGSV, AKI: 2014
2	The total amount of **protein** per sample shall be	<100 µg	RKI guideline: 2012
3	The total amount of **protein** per sample shall be	<200 µg	EN ISO 15883-1: 2006
4	The amount of **protein/cm²** shall be	<6.4 µg/cm²	AAMI TIR 30: 2011 Alfa et al AJIC 1999
5	The amount of **protein/cm²** shall be	<3.0 µg/cm²	Guideline DGKH, DGSV, AKI: 2014
6	The amount of **hemoglobin/cm²** shall be	<2.2 µg/cm²	AAMI TIR 30: 2011 Alfa et al AJIC 1999
7	The total amount of **radioactivity** per sample shall be	<5 cps	SMP Report 11011010605

Table 1: Acceptance criteria for evaluating the cleaning success of reusable medical devices

The sponsor is responsible for the selection and application of suitable acceptance criteria for the cleaning efficacy as well as the interpretation of the data.

For the present investigation the acceptance criteria 1, 2, 5, 6 und 7 were applied.

2 Summary

The objective of this project was to examine the cleaning behavior of the samples listed in Table 4 in an automated cleaning process.

The samples were contaminated under conditions that simulate real use. The contamination was held intentionally high with locations hard to clean being especially in the focus. Thus, a worst case scenario was created.

The test soil was labelled radioactively to both quantify and localize the amount of contamination and identify and localize residues of the test soil after the cleaning process.

Residual protein on and in the samples were extracted with 1% SDS-solution and quantified spectrophotometrically utilizing a modified OPA assay.

In addition, a visual, semi-quantitative hemoglobin test strip (Combur^{3} Test® E; Roche; REF 11896857191) was used to determine the amount of hemoglobin in the extracts.

The examination was performed according to the relevant standards and guidelines (see 11. "References and Standards").

The laboratory of SMP GmbH is accredited to perform examinations of the cleanability of medical devices according to DIN EN ISO / IEC 17025:2005 and guidelines 93/42/EEC and 90/385/EEC (Certificate ID: D-PL-17769-01-01).

3 Conclusion

At the end of the cleaning process there was **no soil visible** on the samples under normal illumination.

The results of the examination are summarized and presented in Table 2 and Table 3.

Criterion		Average	Maximum	Acceptance level
protein	**[µg]**	**50**	**54**	**< 100**
protein/cm²	**[µg / cm²]**	**0.51**	**0.67**	**< 3.0**
hemoglobin/cm²	**[µg / cm²]**	**< 0.05**	**< 0.05**	**< 2.2**
Radioactivity (assembled samples)	**[cps]**	**0.15**	**0.4**	**< 5**

Table 2: Summary of the results after cleaning **Global – Limiter with silicone handle**

Criterion		Average	Maximum	Acceptance level
protein	**[µg]**	**11**	**17**	**< 100**
protein/cm²	**[µg / cm²]**	**0.10**	**0.15**	**< 3.0**
hemoglobin/cm²	**[µg / cm²]**	**0***	**0***	**< 2.2**
Radioactivity (assembled samples)	**[cps]**	**0.15**	**0.4**	**< 5**

Table 3: Summary of the results after cleaning **Self – Retaining Screwdriver**

* The result was below the limit of detection as given in Chapter 8.5

The applied acceptance criteria 1, 2, 5, 6 and 7 described in Chapter 1 were fulfilled.

4 Samples under Examination

Sample No.	Identification	Surface Area [cm²]	REF	LOT	Figure
13616-01-1	Global – Limiter with silicone handle 0.5 Nm, Fa. Weber	98,46 cm²	W30-039-U	9150986	1
13616-01-2					
13616-01-3	Global – Limiter with silicone handle 1.3 Nm, Fa. Weber				
13616-01-4	Global – Limiter with silicone handle 1.5 Nm, Fa. Weber				
13616-02-1	Self – Retaining Screwdriver T15 with AO coupling	111,72 cm²	-	-	2
13616-02-2					
13616-02-3					
13616-02-4	Self – Retaining Screwdriver T15	111,72 cm²	703580/2	KMW250701	
13616-01-1-X (X=1-4)	Assembled Sample	210,18 cm²	-	-	3

Table 4: Samples examined

The surface area data were provided by the sponsor.

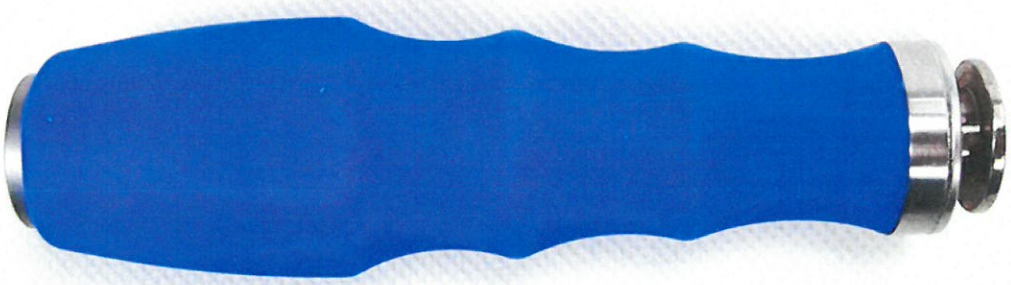


Figure 1: 13616-01-X (X=1-4) = Global – Limiter with silicone handle, REF W30-039-U

Figure 2: 13616-02-X (X=1-4) Self – Retaining Screwdriver

Figure 3: 13616-01-1-X (X=1-4) Assembled Sample

5 Contamination of the Samples

5.1 Composition of the Test Soil

The mixing ratio of the test soil was:

- 10 ml heparinized sheep blood (Acila AG, REF 2132019)
- 100 MBq ^{99m}Tc bound to macro-aggregated albumin (GE Healthcare, REF P722DE)
- 150 µl protamine hydrochloride (MEDA Pharma GmbH & Co. KG, REF 6888914)

Protamin hydrochloride is an antagonist to heparin. It is used to make the test soil coagulable.

5.2 Method of Contamination

The samples were contaminated as described in Table 5. The figures listed therein illustrate the process.

Sample No.	Method of Contamination	Figure
13616-01-1-1 13616-01-1-2 13616-01-1-3	The tip of the assembled instrument was immersed in the test soil. The sample was touched with contaminated gloves. After contamination the self – retaining screwdriver was removed from the global limiter and assembled with contaminated gloves for another time.	4-6
13616-01-1-4	Negative device control / no contamination	-

Table 5: Description of contamination

One sample remained uncontaminated and served as negative device control. This sample was subjected to the same cleaning and extraction procedure as the test devices.

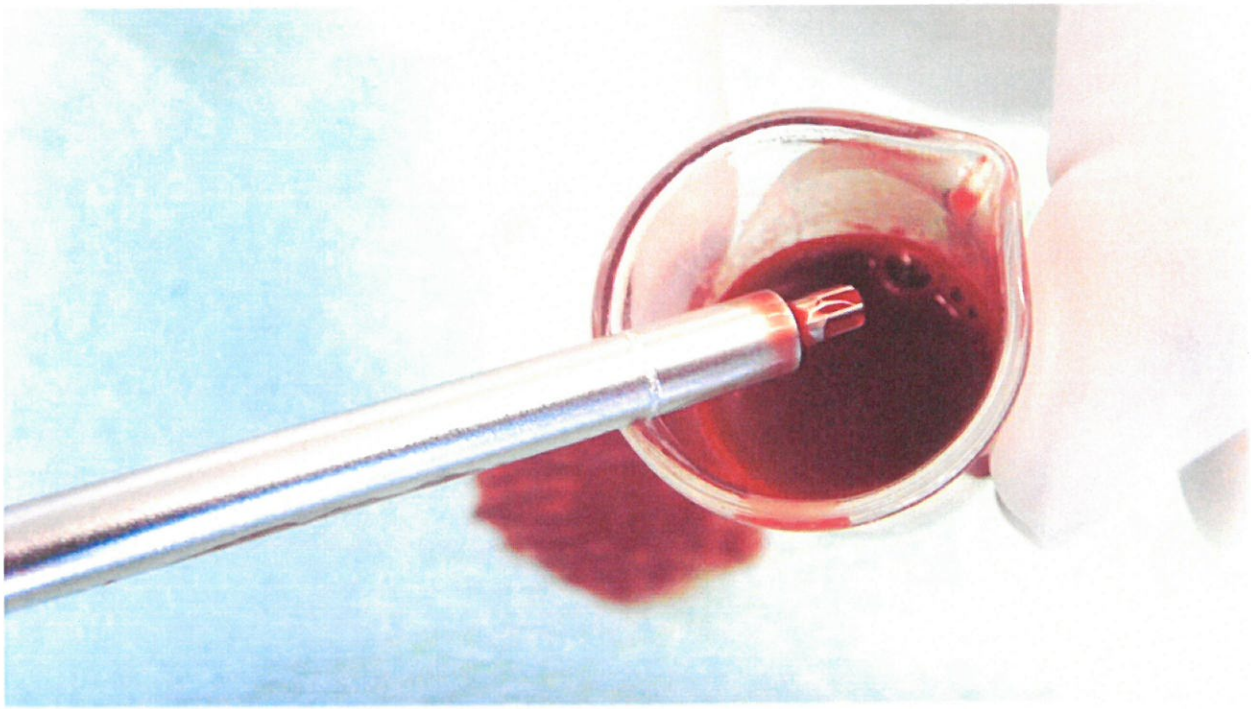


Figure 4: Contamination of a Sample 13616-01-1-X (X= 1, 2 and 3)

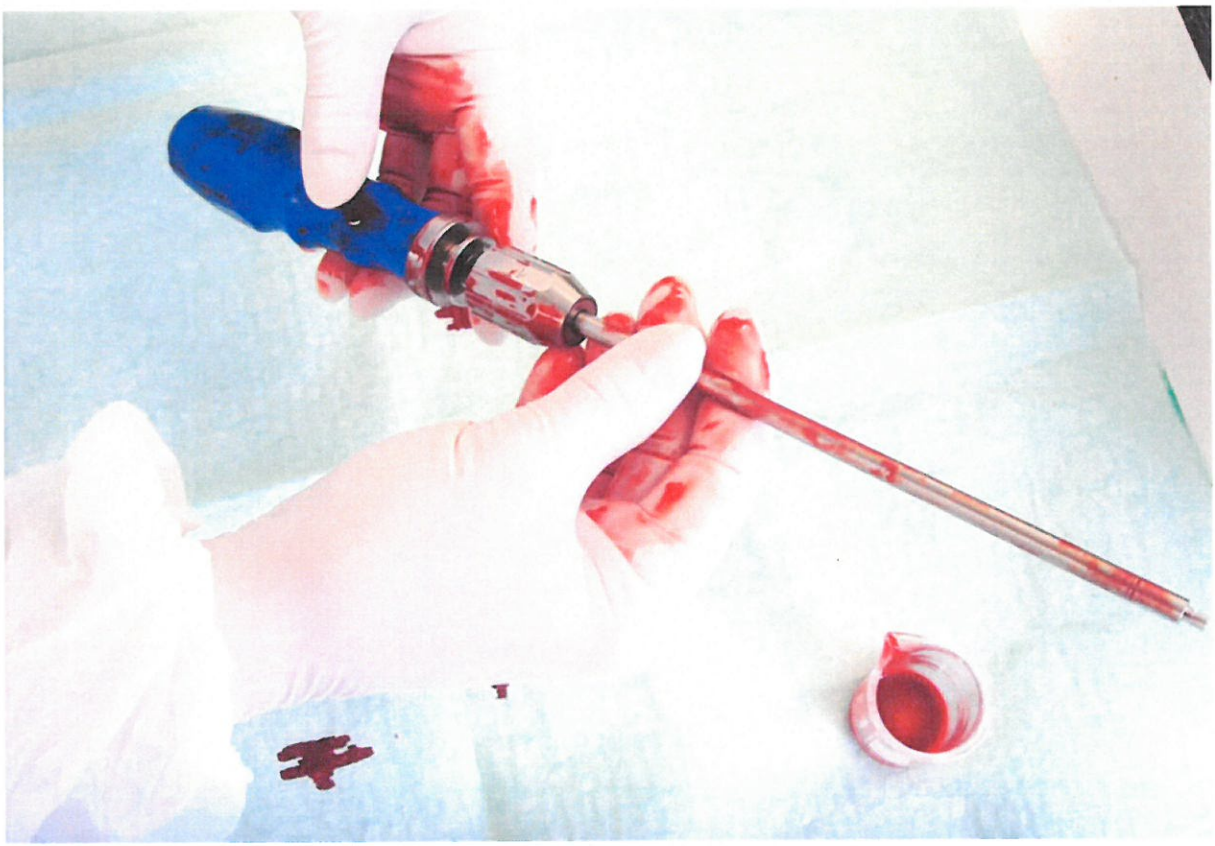


Figure 5: Contamination of an assembled sample 13616-01-1-X (X= 1, 2 and 3)

Figure 6: Contamination of an assembled sample 13616-01-1-X (X= 1, 2 and 3)

After contamination the amount of test soil in and on the samples was measured with the gamma camera and individually evaluated for each sample.

6 Drying of the Samples

After contamination the samples were stored for one hour at ambient conditions to allow the test soil to dry.

7 Cleaning Process

The cleaning process is described in Table 6

Cleaning Step	Description	
Manual Pre-Cleaning	Step 1	The self – retaining screwdriver was removed from the global limiter and both parts of the sample were completely immersed in lukewarm tap water for 5 min. The lumen of the Self – Retaining Screwdriver was filled with water.
	Step 2	The surfaces of the sample was brushed using running tap water with a soft nylon brush (Medisafe Med 100.33) until no residues were visible. The lumen of the self – Retaining Screwdriver was brushed with a suitable bottle brush.
	Step 3	The sample was treated in an ultrasonic bath for 5 min (frequency 35 kHz, room temperature) filled with 0.5 % cleaning solution in desalinated water (neodisher Mediclean Dr. Weigert). Before starting sonication the lumen were filled with cleaning solution.
	Step 4	A water jet gun was used to clean the lumen and gaps for 15s at 2 bar.
Automated Cleaning	▪ 2 min pre-cleaning with cold tap water (16°C±2°C)* ▪ Draining ▪ 5 min cleaning at 55°C with tap water and 0.5 % cleaning solution (neodisher MediClean) ▪ Draining ▪ 3 min rinsing with cold demineralized water (20°C±2°C) ▪ Draining ▪ 2 min rinsing with cold demineralized water (20°C±2°C) ▪ Draining	
Washer-Disinfector	Miele G7836 CD	
Rack	E327 / 1274658	

Arrangement of the samples in the washer-disinfector	 Figure 7: Samples in the washer-disinfector
Cleaner	neodisher MediClean (Chemische Fabrik Dr. Weigert GmbH & Co. KG, REF 42259)
Remarks	The automated cleaning process was aborted prior to the thermal disinfection step (final rinse). *Temperatures given refer to the water inlet of the washer-disinfector. The actual temperatures during the cleaning cycle can be derived from the logger diagram given in Figure 8.

Table 6: Description of the cleaning process

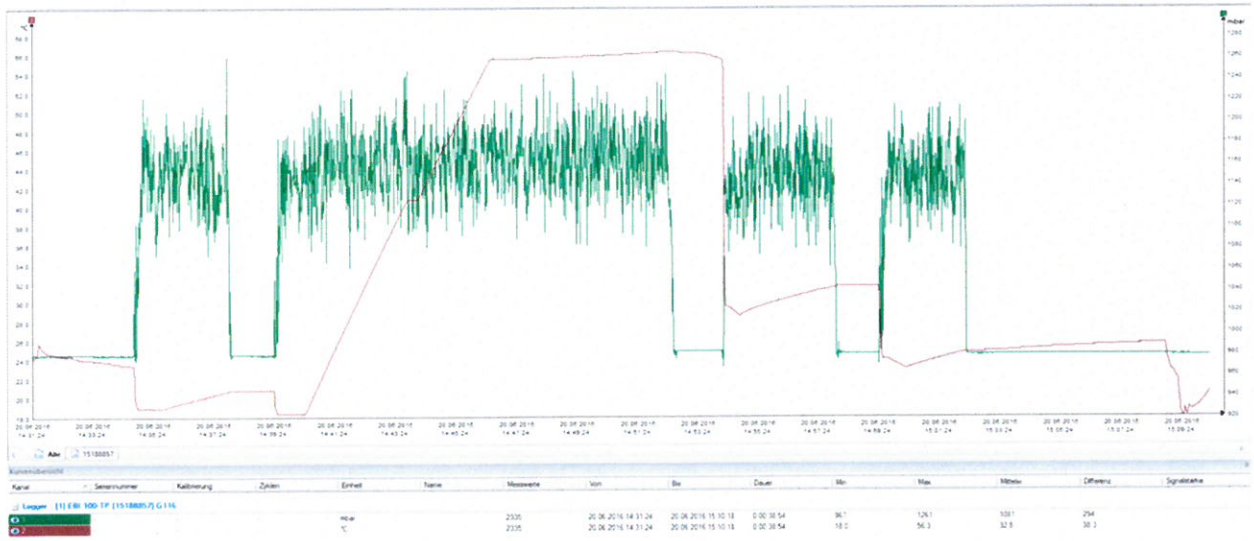


Figure 8: Logger diagram of the cleaning process

Green line: Pressure
Red line: Temperature

8 Quantification of Contamination and Cleaning Efficiency

8.1 Radioactive Labelling of the Test Soil / Radionuclide Method

As described in method MD1.1 in detail, the test soil was radioactively labelled by adding a preparation of human macro-aggregated albumins and metastable Technetium. Radioactive Technetium 99mTc decays with a half-life time of 6.01 hours. The energy of the gamma radiation (140 keV) is high enough to penetrate all materials which are generally used for medical devices and can be detected quantitatively and spatially resolved using a standard gamma camera. Therefore radioactive labelling of the test soil is a method for measuring the amount and distribution of the test soil after the contamination on and in the examined samples both directly and quantitatively. As the measurement does not interfere with the cleaning process, the efficiency of intermediate cleaning steps can be monitored independently from each other.

The amount of radioactivity measured is expressed in counts per second (cps) and is denoted Zr throughout the report.

Extensive examinations in the past proved that a residual amount of radioactivity not higher than 5 cps corresponds with a probability of better than 99% to an amount of residual protein of not higher than 100 µg.

8.2 Extraction of residual soil

The extraction of residual soil from a sample was performed with 5 ml alkalized (pH=11.0) 1% aqueous sodium dodecyl sulfate (Roth, REF CN30.4) solution (SDS).

The extraction is described in Table 8. The figures listed therein illustrate the process.

Step	Method of Extraction	Figure
1	The disassembled sample was placed in a plastic pouch filled with 5 ml SDS solution and closed by sealing. The global limiter and the screwdriver were extracted separately.	9,10
2	The sample was moved in the pouch in order to allow the solution to reach all surfaces and ports.	
3	10 min soaking time.	
4	Steps 2 and 3 were repeated two additional times.	

Table 7: Description of the extraction

Figure 9: Extraction of a sample

Figure 10: Extraction of a sample

8.3 Quantitative Protein Detection – Modified OPA Assay

The modified OPA assay (OPA=ortho-phthaldialdehyde) is a quantitative method to determine the concentration of proteins with free α- and ε-terminal amino (-NH_2) groups in a solution. The OPA assay is based on the chemical reaction of free amino groups with ortho-phthaldialdehyde in presence of a thiol compound to fluorescent isoindoles, which can be detected spectrophotometrically at 340 nm (absorbance maximum).

For each test series, calibration (linear regression) was performed against BSA (bovine serum albumin) standard solutions which were prepared in 1% SDS solution ranging from 0 µg/ml to 50 µg/ml. The standards were mixed in a 1:1 part ratio with the working reagent, incubated for 3 min at 37 °C and the absorbance was read at a wavelength of 340 nm. The absorbance was corrected for its corresponding blank. Each measurement was performed in triplicate.

An exemplary calibration curve is given in the Annex.

- The average limit of quantification was calculated to be 2.2 µg/ml ± 0.9 µg/ml.
- The average limit of detection was calculated to be 0.6 µg/ml ± 0.3 µg/ml.

Results below the limit of detection are equivalent to “not detectable”. Results between the limit of detection and the limit of quantification are estimates.

8.4 Hemoglobin Detection

To determine the amount of hemoglobin in the extracts a visual, semi-quantitative hemoglobin test strip (Combur^{3}Test®E; Roche; REF 11896857191) was used. The peroxidase-like action of hemoglobin and myoglobin specifically catalyzes the oxidation of the indicator by means of the organic hydroperoxide contained in the test paper to give a blue-green coloration. The coloration was compared to standards with a known concentration of hemoglobin (see Table below). The results for this test are given in arbitrary units and translate as follows:

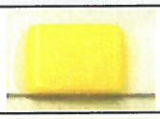
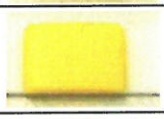
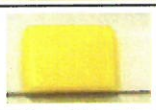
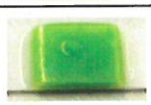
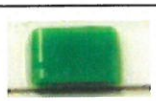
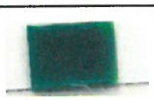
Arbitrary unit	Hemoglobin [µg/ml]	Color
0	No findings	
0	<0.5	
0	<1	
0/1+	<2	
1+/2+	<4	
2+	<8	
3+	<16	
3+/4+	<32	
4+	>32	

Table 8: Correlation of test stick color with hemoglobin concentration

8.5 Determination of the Extraction Efficiency

The examination of positive device controls was used to determine the efficiency of the extraction of soil from the samples. A positive device control is a sample, which is soiled, but not cleaned. The positive device controls were extracted three times as described in Chapter 8.2. The amount of protein which was recovered from the positive device control for each extraction was determined using the protein test as described in Chapter 8.3.

The extraction efficiency was calculated using the following formula:

$$\text{Extraction efficiency [\%]} = \left(\frac{\text{1st Extraction [µg]}}{\text{Sum of all Extractions [µg]}} \right) \text{x 100}$$

Results – Positive Device Controls and Extraction Efficiency Global Limiter

Sample No.	1st Extraction [µg]"	2nd Extraction [µg]"	3rd Extraction [µg]"	Sum of all Extractions [µg]"	Extraction Efficiency [%]
13616-01-1	9693	555	32	10280	94.3
13616-01-2	9433	623	50	10106	93.3
13616-01-3	5553	258	249	6060	91.6
Average					**93.1**

Table 9: Results – Positive Device Controls and Extraction Efficiency

The average extraction efficiency was calculated to be 93.1 % (correction value = 1.07).

Results – Positive Device Controls and Extraction Efficiency Self – Retaining Screwdriver

Sample No.	1st Extraction [µg]"	2nd Extraction [µg]"	3rd Extraction [µg]"	Sum of all Extractions [µg]"	Extraction Efficiency [%]
13616-02-1	6799	258	12	7069	96.2
13616-02-2	5842	226	11	6079	96.1
13616-02-3	6545	492	30	7067	92.6
Average					**95.0**

Table 10: Results – Positive Device Controls and Extraction Efficiency

The average extraction efficiency was calculated to be 95.0 % (correction value = 1.05).

9 Results and Data Collection Sheets

9.1 Characterization of the Test Soil (Positive Sample Control)

20 µl of test soil were examined for the detectable amount of protein and the measurable radioactivity contained.

Run	Sample No.	Protein	Radioactivity	Ratio Protein / Radioactivity
		[µg] per 20µl test soil	[cps] per 20µl test soil	[µg] per [cps]
1	13616-REF	2598	8.1	321
2	13616-REF	2384	8.0	298
3	13616-REF	2250	6.8	331

Table 11: Characterization of the Test Soil (Positive Sample Control)

9.2 Results - Radionuclide Method and Visual Inspection Assembled Sample

Run	Sample No.	After Contamination	After Cleaning	
		Zr [cps]	Zr [cps]	Visual Inspection
1	13616-01-1	61.0	0.1	No Findings
	13616-01-2	52.4	0.0	No Findings
	13616-01-3	58.6	0.2	No Findings
2	13616-01-1	61.3	0.4	No Findings
	13616-01-2	48.9	0.2	No Findings
	13616-01-3	60.3	0.1	No Findings
3	13616-01-1	57.7	0.1	No Findings
	13616-01-2	56.4	0.1	No Findings
	13616-01-3	76.3	0.2	No Findings

Table 12: Results - Radionuclide Method and Visual Inspection

9.3 Results – Modified OPA Assay

9.3.1 Global Limiter

The results of the quantitative protein detection [µg/sample] and [µg/cm^2] as presented in the table below are taking into account the average extraction efficiency of 93.1% (correction value = 1.07).

Measurements made with unused extraction fluid are used as negative sample controls.

Run	Sample No.	µg/ml	µg/sample	µg/cm^2
1	13616-01-1	9.0	48	0,49
	13616-01-2	9.2	49	0,50
	13616-01-3	10.1	54	0,55
	13616-01-4 (negative device control)	7.2	39	0,40
	13616-SDS (negative sample control)	-	N/A	N/A
2	13616-01-1	11.1	60	0,61
	13616-01-2	10.6	57	0,58
	13616-01-3	12.4	66	0,67
	13616-01-4 (negative device control)	7.3	40	0,41
	13616-SDS (negative sample control)	-	N/A	N/A
3	13616-01-1	8.9	47	0,48
	13616-01-2	10.1	54	0,55
	13616-01-3	11.6	62	0,63
	13616-01-4 (negative device control)	3.5	19	0,19
	13616-SDS (negative sample control)	0.3	1	N/A

Table 13: Results - Modified OPA Assay

9.3.2 Self – Retaining Screwdriver

The results of the quantitative protein detection [µg/sample] and [µg/cm^2] as presented in the table below are taking into account the average extraction efficiency of 95.0 % (correction value = 1.05).

Measurements made with unused extraction fluid are used as negative sample controls.

Run	Sample No.	µg/ml	µg/sample	µg/cm^2
1	13616-02-1	3.0	16	0,14
	13616-02-2	1.4	7	0,06
	13616-02-3	3.2	17	0,15
	13616-02-4 (negative device control)	1.2	6	0,05
	13616-SDS (negative sample control)	-	N/A	N/A
2	13616-2-1	2.1	11	0,10
	13616-02-2	1.7	9	0,08
	13616-02-3	1.5	8	0,07
	13616-02-4 (negative device control)	1.0	5	0,04
	13616-SDS (negative sample control)	-	N/A	N/A
3	13616-02-1	2.0	11	0,10
	13616-02-2	2.2	12	0,11
	13616-02-3	1.5	8	0,07
	13616-02-4 (negative device control)	0.7	4	0,04
	13616-SDS (negative sample control)	0.3	1	N/A

Table 14: Results - Modified OPA Assay

9.4 Results – Hemoglobin Detection

9.4.1 Global Limiter

Run	Sample No.	Arbitrary unit	µg/ml	µg/sample	µg/cm²
1	13616-01-1	1+	< 1	< 5	< 0,05
	13616-01-2	1+	< 1	< 5	< 0,05
	13616-01-3	1+	< 1	< 5	< 0,05
	13616-01-4 (negative device control)	0	No Finding	No Finding	No Finding
2	13616-01-1	1+	< 1	< 5	< 0,05
	13616-01-2	1+	< 1	< 5	< 0,05
	13616-01-3	1+	< 1	< 5	< 0,05
	13616-01-4 (negative device control)	0	No Finding	No Finding	No Finding
3	13616-01-1	1+	< 1	< 5	< 0,05
	13616-01-2	1+	< 1	< 5	< 0,05
	13616-01-3	1+	< 1	< 5	< 0,05
	13616-01-4 (negative device control)	0	No Finding	No Finding	No Finding

Table 15: Results – Hemoglobin Detection

9.4.2 Self – Retaining Screwdriver

Run	Sample No.	Arbitrary unit	µg/ml	µg/sample	µg/cm²
1	13616-02-1	0	No Finding	No Finding	No Finding
	13616-02-2	0	No Finding	No Finding	No Finding
	13616-02-3	0	No Finding	No Finding	No Finding
	13616-02-4 (negative device control)	0	No Finding	No Finding	No Finding
2	13616-02-1	0	No Finding	No Finding	No Finding
	13616-02-2	0	No Finding	No Finding	No Finding
	13616-02-3	0	No Finding	No Finding	No Finding
	13616-02-4 (negative device control)	0	No Finding	No Finding	No Finding
3	13616-02-1	0	No Finding	No Finding	No Finding
	13616-02-2	0	No Finding	No Finding	No Finding
	13616-02-3	0	No Finding	No Finding	No Finding
	13616-02-4 (negative device control)	0	No Finding	No Finding	No Finding

Table 16: Results – Hemoglobin Detection

10 Photo Documentation

The following exemplary figures taken from one run of the examination show the samples 13616-01-X (X=1–3) Global Limiter und 13616-02-1-X (X=1-3) Self – Retaining Screwdriver on top of the gamma camera. All runs performed were documented in the same way.

Fig.	Description
11	Clean samples on the gamma camera
12	Samples after contamination [1)]
13	Sample after pre-cleaning / step 4 [1)]
14	Samples after cleaning [1)]
Note:	Randomly distributed grey points are caused by background radiation.

[1)] Superposition of a conventional photograph with a false color representation of the measured radioactivity

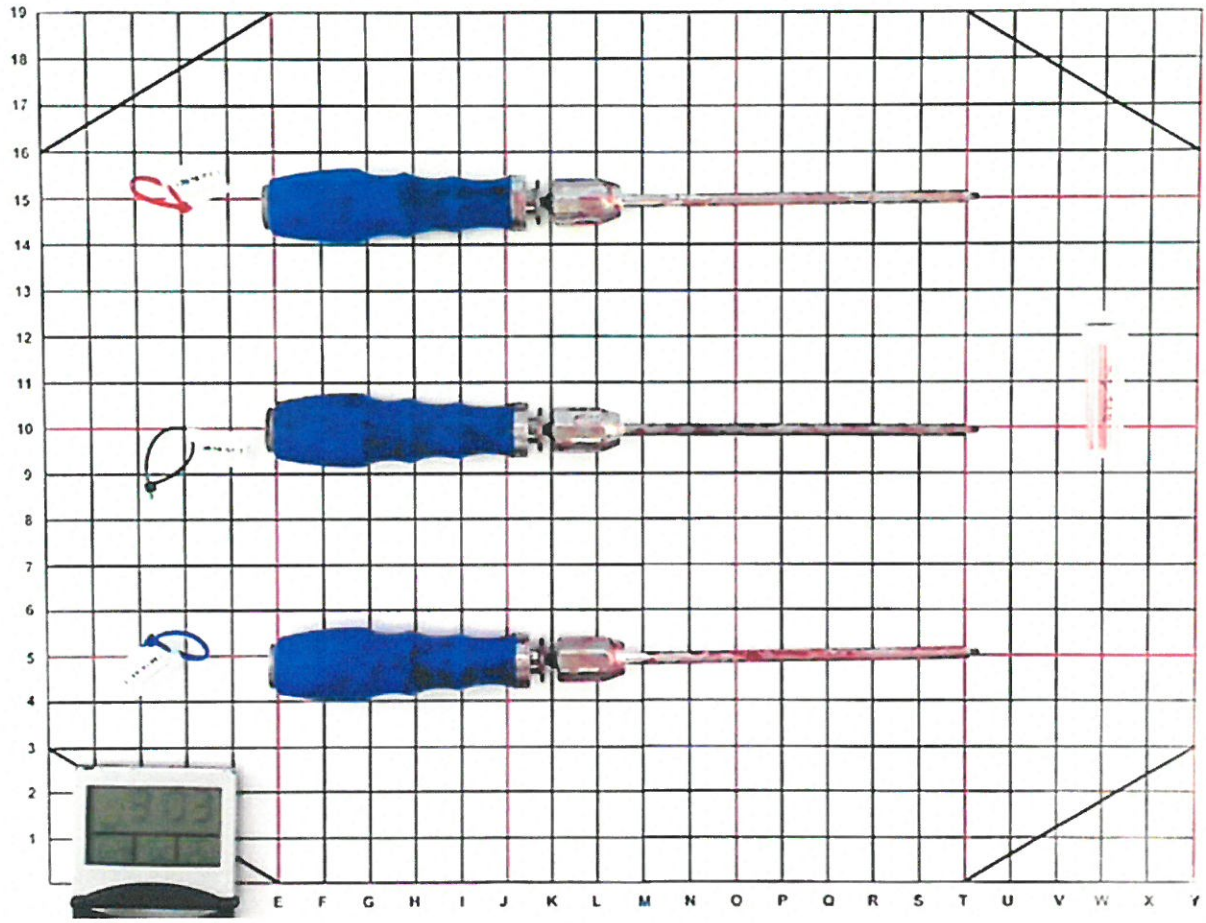


Figure 11: Clean samples assembled 13616-01-1-X (X=1-3):

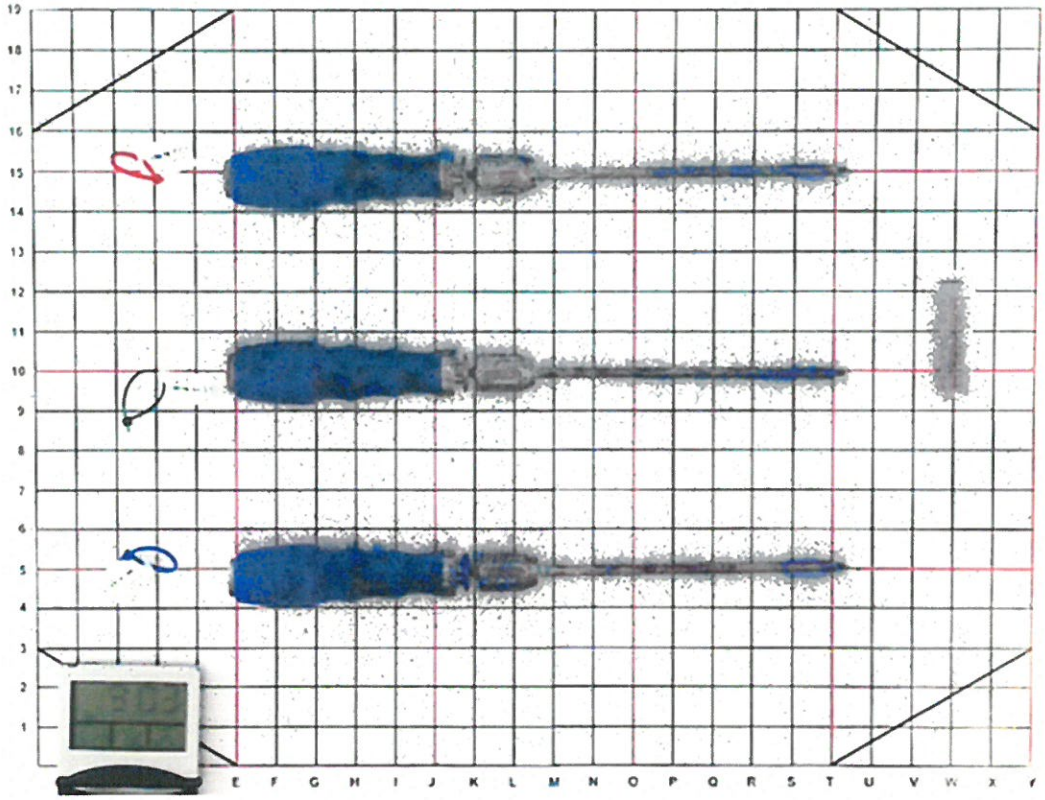


Figure 12: Samples assembled 13616-01-1-X (X=1-3) after contamination

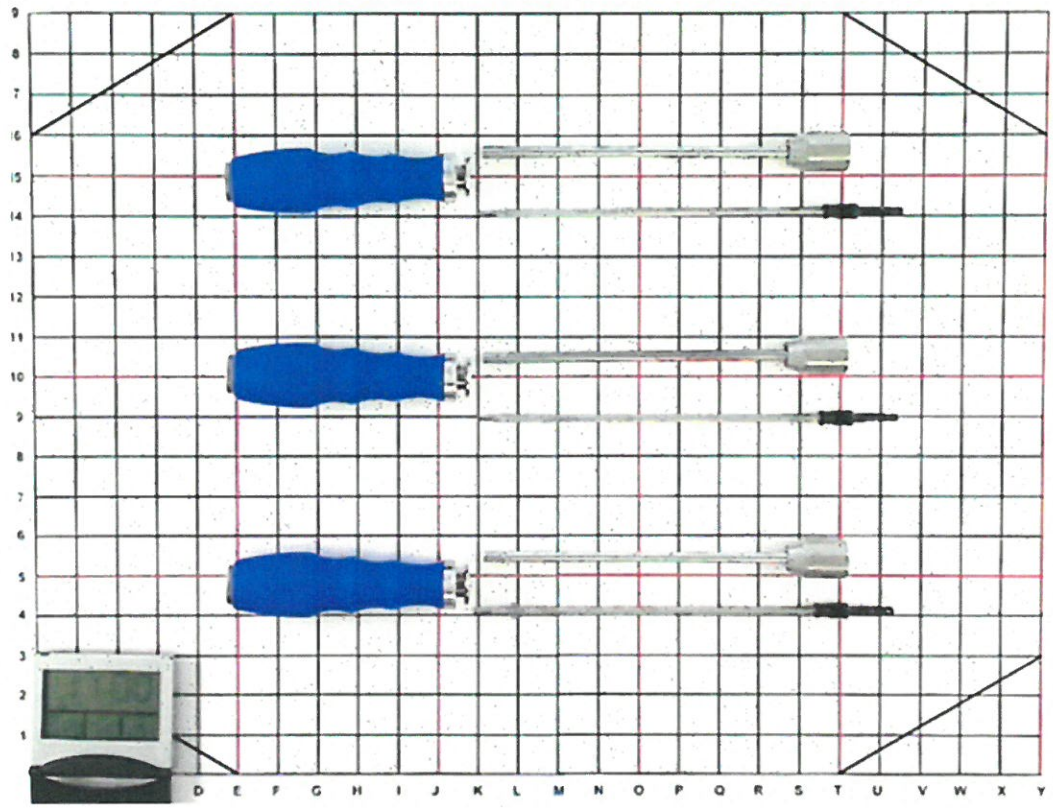


Figure 13: Samples disassembled 13616-01-1-X (X=1-3) after step 4

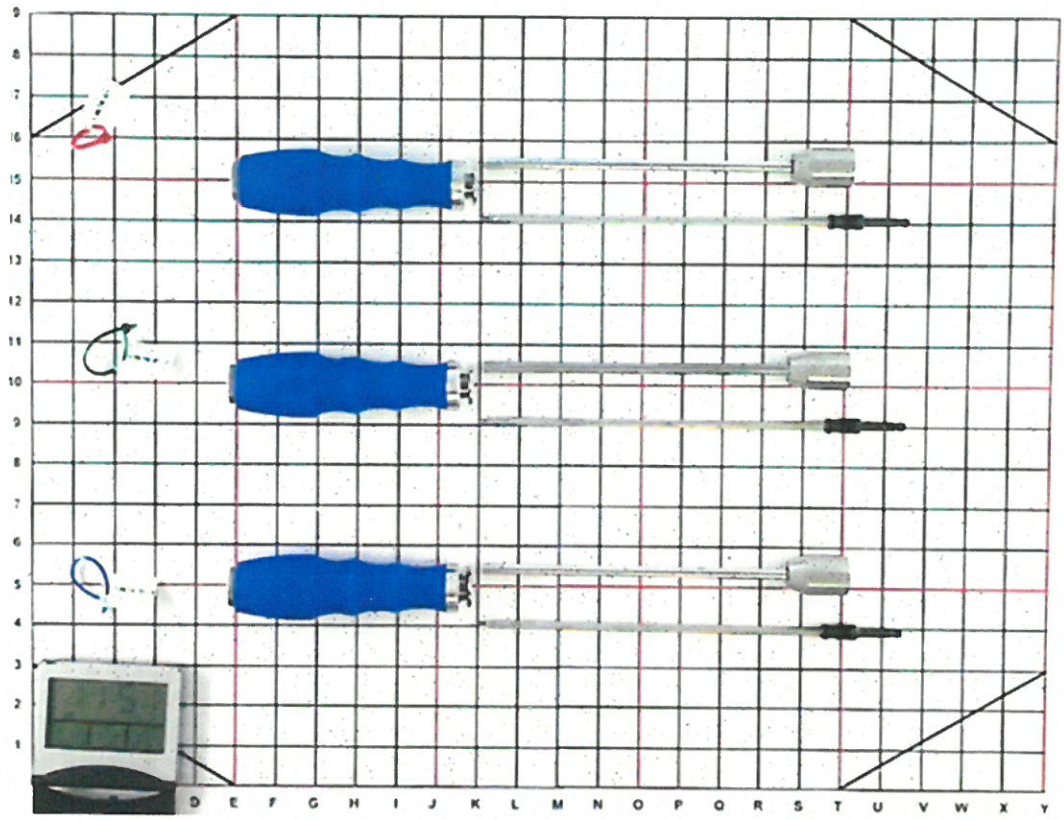


Figure 14: Samples disassembled 13616-01-1-X (X=1-3) after cleaning

11 References and Standards

Identification	Title
AAMI TIR 12	Publication date: 2010 Designing, testing, and labeling reusable medical devices for reprocessing in healthcare facilities: A Guide for Device Manufacturers
AAMI TIR 30	Publication date: 2011 TIR 30 "A compendium of processes, materials, test methods, acceptance criteria for cleaning reusable medical devices
AAMI ST-81	Publication date: 2004 Sterilization of medical devices – Information to be provided by the manufacturer for the processing of resterilizable medical devices
ISO 17664	Publication date: 2004-03 Sterilization of medical devices - Information to be provided by the manufacturer for the processing of resterilizable medical devices
EN ISO 15883-1	Publication date: 2009 Washer/Disinfectors - Part 1: General requirements, terms and definitions and tests; German version EN ISO 15883-1:2006
TS/ISO 15883-5	Publication date: 2006 Test soils and methods for demonstrating cleaning efficacy of washer disinfectors
Guideline DGKH, DGSV, AKI: 2014	Publication date: 2014 Guideline of DGKH, DGSV und AKI for the validation and routine control of automated cleaning and disinfection processes for thermostable medical devices and principles of device selection.
Guideline DGKH, DGSV, AKI: 2013	Publication date: 2013 Guideline of DGKH, DGSV und AKI for the validation of manual cleaning and chemical disinfection of medical devices.
RKI guideline: 2012	Publication date: 2012 Requirements on hygiene for reprocessing of medical devices, Robert-Koch Institute, Germany
Alfa et al AJIC 1999	Alfa MJ, DeGagne P, Olson N. Worst-case soiling levels for patient-used flexible endoscopes before and after cleaning. Am J Infect Control 1999; 27:392-401.
FDA Guidance Document Reprocessing 2015	Publication date: 2015 Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling Guidance for Industry and Food and Drug Administration Staff

Internal Documents	
MD1.1	Examination and Validation of the Cleanability of Medical Devices using radioactive Markers
MD1.2	Examination and Validation of the Cleanability of Medical Devices using Protein Detection Methods
SOP03	Production of radioactively labelled test soil
SOP04	Contamination with radioactively labelled test soil
SOP05	Cleaning if radioactively labelled test soil is used
SOP36	Measurement with the Gamma camera
SOP37	Data evaluation Gamma camera
SOP14	Extraction of protein
SOP18	Spectrophotometric protein quantification via a modified OPA assay
SOP19	Spectrophotometric protein quantification via a Biuret / BCA assay
SOP32	Determination of the hemoglobin content
SOP35	Production of reagents for protein quantification
SMP Report 11011010605	Validation of the acceptance criteria of the Radionuclide Method as performed by SMP GmbH with respect to the total amount of residual protein

12 Abbreviations

Abbreviation	Description
MD	Description of accredited Method
SOP	Standard Operation Procedure
DGSV	German Society Sterile Supply
DGKH	German Society for Hospital Hygiene
RKI	Robert Koch Institute
AKI	Working Group Instrument Reprocessing
FDA	Food and Drug Administration
OPA	ortho-phthalaldehyde
BCA	bicinchoninic acid
BSA	bovine serum albumin
SDS	sodium dodecyl sulfate
NDC	negative device control
NSC	negative sample control
PDC	positive device control
PSC	positive sample control
W/D	washer / disinfector
DS / VE	desalinated (water)
N/A	not applicable

Archiving:

The original report is property of the sponsor.
One copy of the report including raw data remains at SMP.

----------End of the report----------

13 Annex

13.1 Example data of an OPA calibration

Analytical limits taken from the calibration function

Limit of detection	0.6	µg/ml (BSA equivalent)
Limit of quantification	2.0	µg/ml (BSA equivalent)

Calibration points

No.	Concentration [µg/ml] BSA	Response1	Response2	Response3	Mean Response	Standard Deviation
1	5.00	0.0595	0.0589	0.0588	0.0590	0.0004
2	10.00	0.0737	0.0728	0.0727	0.0731	0.0006
3	15.00	0.0866	0.0865	0.0852	0.0861	0.0008
4	20.45	0.1034	0.1020	0.1014	0.1022	0.0010
5	30.00	0.1288	0.1284	0.1273	0.1282	0.0008
6	34.62	0.1410	0.1399	0.1374	0.1394	0.0018
7	40.91	0.1589	0.1583	0.1579	0.1584	0.0005
8	45.00	0.1717	0.1698	0.1679	0.1698	0.0019
9	50.00	0.1843	0.1829	0.1826	0.1832	0.0009
10	0.00	0.0476	0.0463	0.0458	0.0466	0.0009

Specifications

Linear Regression y=a*x+b

Slope **a**	0.0027
Intercept **b**	0.0457
Correlation coefficient **R**	0.9999
Standard error of estimate **Sy**	0.001
Standard error of procedure **Sx**	0.253
Number of measurements **n**	3
Result uncertainty	33.33%
Probability of error	1.00%

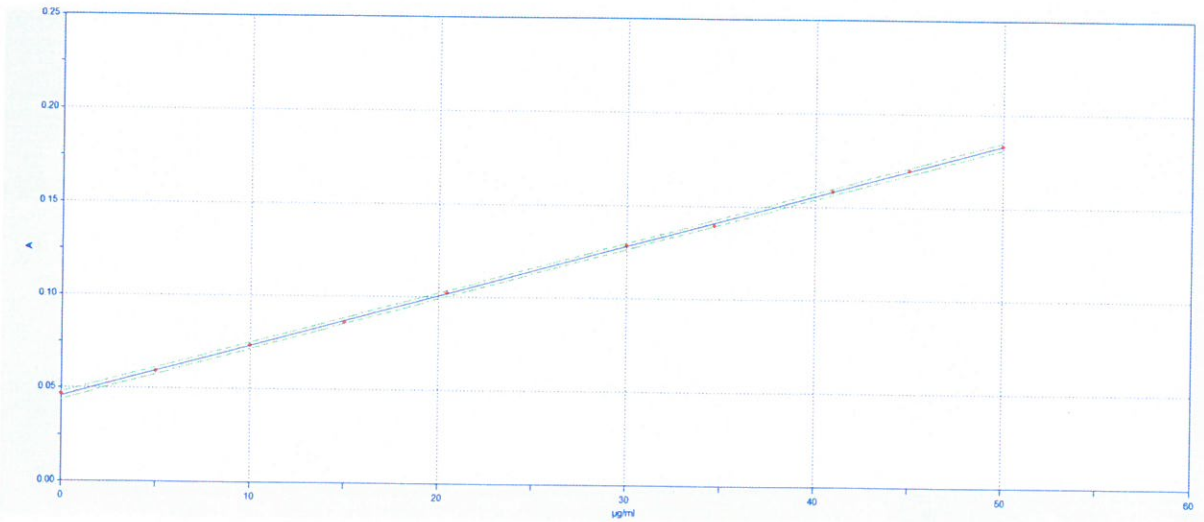


Figure 15: OPA calibration function (linear regression) with confidence interval