Medizinische Fakultät Carl Gustav Carus
Reformfakultät des Stifterverbandes
für die Deutsche Wissenschaft
Harvard Medical International Associated Institution

TECHNISCHE
UNIVERSITÄT
DRESDEN

Institut für Medizinische Mikrobiologie und Hygiene
Direktor: Prof. Dr. med. E. Jacobs

TU Dresden • Medizinische Fakultät • Felscherstraße 74 • 01307 Dresden

Akkreditiert durch
Zentralstelle der Länder
für Gesundheitsschutz
bei Arzneimitteln
und Medizinprodukten
ZLG-P-481.04.07

TECNO-GAZ S.p.A. a socio unico
Strada Cavalli, 4
43038 Sala Baganza
Parma
Italia

Tel.: (0351) 458 2948
Fax: (0351) 458 5729
Lutz.Jatzwauk@
uniklinikum-dresden.de

Dresden, 20 July 2014

FINAL REPORT

Tests of the cleaning and disinfection of medical instruments in a processing device of the type MultiSteril in combination with Multisteril CD in due consideration of the requirements of EN 15883

Client:
TECNO-GAZ S.p.A. a socio unico
Strada Cavalli, 4
43038 Sala Baganza
Parma
Italia

Head of the study: Prof. Dr. Lutz Jatzwauk

Involved employees:
- Sylvia Sadowsky
- Katharina Rust

Postanschrift:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:
Mtz
Fiedlerstraße 42
Raum: B.10.040

Realisation of the technical and microbiological tests and study management:

Dresden University of Technology
Faculty of Medicine
Institute for Medical Microbiology and Hygiene
Fetscherstr. 74
01307 Dresden

Realisation of the protein analyses:

SMP GmbH
Hechingerstraße 262
72072 Tübingen

Test period: 01 May 2014 to 01 July 2014

Quality assurance:

These tests were conducted in compliance with the quality management system according to DIN EN ISO 9001 (Quality management systems - Requirements; 2008) as well as DIN EN ISO 17025 (General requirements for the competence of testing and calibration laboratories; 2005). The accreditation was granted by the Central Authority of the States for Health Protection with Regard to Medicinal Products and Medical Devices (ZLG) under registration number ZLG-P-481.04.07.-01.

Number of report copies:

Client: 1 copy (original)
Test laboratory: 1 copy (original)

Table of contents

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

1. Aim of the tests

The tests performed were supposed to evaluate the cleaning and disinfection effectiveness of medical instruments in an automatic treatment method in the processing device ("multifunctional tray") MultiSteril by Company TECNO-GAZ Industries (Sala Baganza, Parma, Italy) in combination with the cleaning and disinfection agent Multisteril CD (ALPRO MEDICAL GMBH, St. Georgen, Germany). For this purpose, contaminated instruments or test specimens were processed in the laboratory using this method and then examined for surviving micro-organisms or residual protein. The method is considered suitable if:

- a reduction of the bioburden (test germ: *Enterococcus faecium*) by at least 5 log levels has been achieved.
- the residual contamination (amount of protein elutable from the surface of the instruments) complies with the acceptance criteria of the "Guideline by DGKH, DGSV and AKI for the validation and routine monitoring of automatic cleaning and thermal disinfection processes for medical devices and on the principles of device selection" of October 2008.

The acceptance criteria for the amount of protein after cleaning provide for:

1.1. no contamination is optically visible after the processing.
1.2. protein per test specimen falls below a guidance level (warning value) of 100 µg.
1.3. protein per test specimen does not exceed a limit of 200 µg.

Postanschrift:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:
Mtz
Fiedlerstraße 42
Raum: B.10.040

2. Examination methods

2.1. Examination of the cleaning effect

The contamination of the instruments to be examined was achieved in compliance with Appendix 4 of the "Guideline by DGKH, DGSV and AKI for the validation and routine monitoring of automatic cleaning and thermal disinfection processes for medical devices and on the principles of device selection" of October 2008 using coagulable blood. To produce coagulable blood, sterile heparinised sheep blood was mixed with protamine sulphate solution in a ratio of 100: 1 and immediately used for the contamination. Pursuant to the above-mentioned guideline, haemostats according to Crile (figure 1) were used as germ carriers that were specifically contaminated in the hinge area. The initial contamination of the untreated test specimens was 10,000 to 15,000 µg of residual protein (BSA equivalent).

Figure 1: haemostat according to Crile

The protein test in the SDS eluate after the treatment was conducted using the modified OPA method (ISO 15883-1:2006 Annex C). This test method for blood contamination is based on the determination of free o-phthaldialdehyde (OPA)-sensitive amino groups of the blood proteins. In the presence of a thiol component, OPA reacts with free amino groups of the blood proteins (terminal a and e amino groups) to form fluorescent substances which can be detected photometrically.

Postanschrift:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:
Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:
Mtz
Fiedlerstraße 42
Raum: B.10.040

2.2. Examination of the disinfection effect

The disinfection effect was determined according to the guideline of the Federal Health Office on the examination of thermal disinfection methods in cleaning automates (Federal Health Gazette 23, 364-67, 1980). Stainless steel screws (figure 2) act as germ carriers. In deviation from the method described there, coagulable blood was used in these tests.

Figure 2: stainless steel screw as germ carrier for enterococcus faecium

Enterococcus faecium ATCC 6057 was incubated for 48 hours at 36 ± 1.0 °C on Columbia blood agar, elutriated using a physiological salt solution, centrifuged for 10 minutes at 8000 rpm and re-suspended in physiological salt solution. The suspension was then tuned to a bacteria concentration of approximately 108 CFU/ml. Sterile heparinised sheep blood (ACILA AG, Möhrfelden) was mixed with protamine sulphate solution in a ratio of 100:1, then mixed with four times the suspension of enterococcus faecium and used immediately for the contamination. For the elution of *Enterococcus faecium*, the instruments are treated on the analogy of the above-mentioned elution of protein residue. A sterile physiological salt solution in addition of 0.1% of tween 80, 0.3% of saponine, 0.1% of histidine and 0.1% of cysteine (for the disinhibition of possibly present disinfectant residues) serves as rinsing solution. The rinsing solution is filtered in aseptic conditions using cellulose membrane filters (pore diameter 0.2 micrometres) and the filters are placed on Columbia blood agar. The incubation of *Enterococcus faecium* was realised for 48 hours at 36 °C. Then the grown colony-forming units (CFU) with generic morphology were counted. The number of colonies of the suspension used for the contamination as well as the number of colonies of the rinsing solutions are calculated. Determining the bioburden before and after the treatment serves to calculate the reduction in colony numbers as a measure of the disinfection achieved.

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

2.3. Cleaning and disinfection methods

Treating the instruments or germ carriers was done in a processing device of the type "MultiSteril" by Company TECNO-GAZ Industries. The instruments were cleaned by means of a chemical cleaning solution with the aid of ultrasound (39 KHz, max. 1 KW). In order to disinfect the instruments, a chemo-thermal disinfection method is used. According to the manufacturer information, the device is operated with the treatment phases disinfection, cleaning, rinsing and drying in cycle A (heating in the context of cleaning, no sweeping function, temperature 30°C, exposure time 20 minutes). The product Multisteril CD (ALPRO MEDICAL GMBH, Germany) in a concentration of 0.5 or 1% was used as disinfection and cleaning agent. The metering was automatically effected by the device. Parallel tests using water without disinfectant were conducted for checking purposes. The process parameters cavitation and temperature, essential for the cleaning effectiveness, were measured. The device was operated using water from the water supply system of the city of Dresden (8 to 15 °dH). The test specimens (Crile haemostats) were exposed in the device according to figure 3.

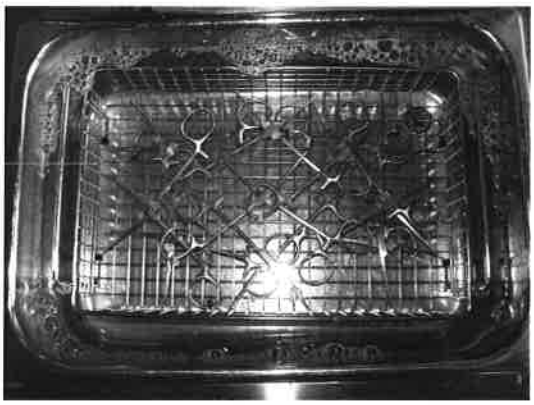

Figure 3: Crile haemostats after positioning in the processing device

The test specimens for the microbiologic examinations (stainless steel screws) were exposed to the processing method in the sieve insert supplied by the manufacturer (figure 4).

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

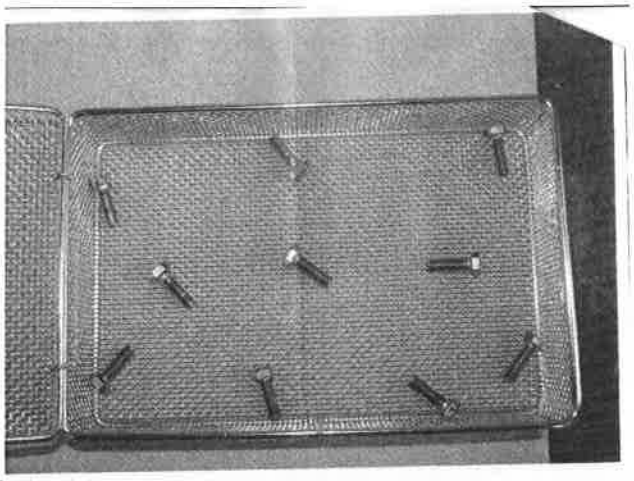

Figure 4: stainless steel screws before exposure in the processing device

2.4. Determination of the cavitation

In ultrasonic baths, the cavitation in addition to the micro-flow is the decisive component for the cleaning of medical devices. It is therefore necessary to standardise the cavitation. It depends on the frequency and intensity of the ultrasound used, on the type of liquid in the ultrasonic bath and its temperature. One method for the quantitative proof of the cavitation is to measure the material erosion on soft metals immersed into the liquid to be examined in an ultrasonic field. The material erosion due to cavitation results in a loss of mass on the test specimen. If you use aluminium foil, the cavitation will become apparent by the formation of holes in the foil. The examination was conducted in terms of quantity following the Australian Standard AS 2773 (Ultrasonic Cleaners For Health Care facilities, Standards Association of Australia, Australia, 1999).

A 30µm aluminium foil (neolab Migge, item no. 1-6597) served as test foil that was treated with ultrasound for 5 minutes.

2.5. Determination of temperatures during the treatment

Time and temperature in the liquid were measured during the treatment with ultrasound using wireless data loggers (ebro Elektronik GmbH, Ingolstadt, Germany). The accuracy of measurement of the loggers was +/- 0.3 °C.

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

3. Results

3.1. Determination of the cavitation

Liquid in the device Multisteril CD 0,5%	Mass of the foil in mg		Mass after / before ultrasonic treatment in %
	before treatment	after treatment	
1	1115	1092	97,9
2	1076	1059	98,4
3	1033	1009	97,7
4	1115	1093	98,0
5	1136	1134	99,8
6	1067	968	90,7
7	1094	1020	93,2
8	1051	1051	100
9	1100	1094	99,5
10	1108	1003	90,5
Mean			96,6
Liquid in the device Multisteril CD 1,0%	**Mass of the foil in mg**		**Mass after / before ultrasonic treatment in %**
	before treatment	**after treatment**	
1	1075	1070	99,5
2	1082	1081	99,9
3	1061	1060	99,9
4	1071	1071	100
5	1081	1076	99,5
6	1079	1037	96,1
7	1113	1108	99,6
8	1090	1071	98,3
9	1101	1074	97,5
10	1062	1029	96,8
Mean			98,7
Liquid in the device Water	**Mass of the foil in mg**		**Mass after / before ultrasonic treatment in %**
	before treatment	**after treatment**	
1	1101	1092	99,1
2	1061	1045	98,5
3	1103	1103	100
4	1088	1075	98,8
5	1109	1103	99,4
6	1103	1091	98,9
7	1085	1083	99,8
8	1072	1063	99,2
9	1071	1042	97,3
10	1074	1066	99,3
Mean			99,0

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

Due to the cavitation, the mass of the aluminium foil is reduced after the treatment in the ultrasonic bath. The average loss of mass is 1% in tap water, 1.3% in 1% Multisteril CD and 3.4% in 0.5% Multisteril CD. The disinfectant thus has no negative influence on the cavitation (compared to water).

3.2. Temperature profile in the processing device

The temperature profile in the device is shown in figure 5. The temperature of the cleaning and disinfection agent stated by the manufacturer is supposed to be 30°C in the cleaning phase.

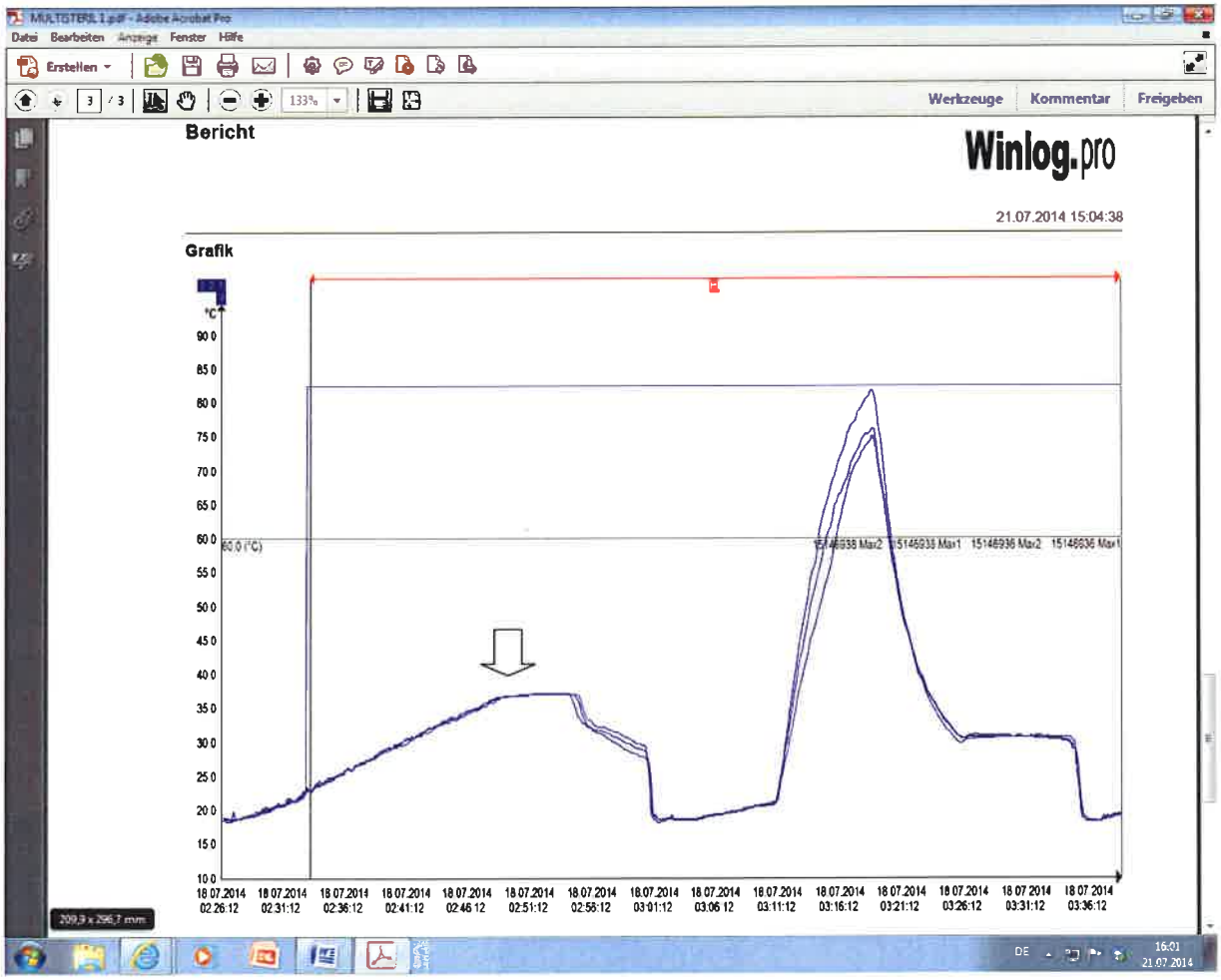

Figure 5: temperature profile of the liquid in the processing device (batch I)

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

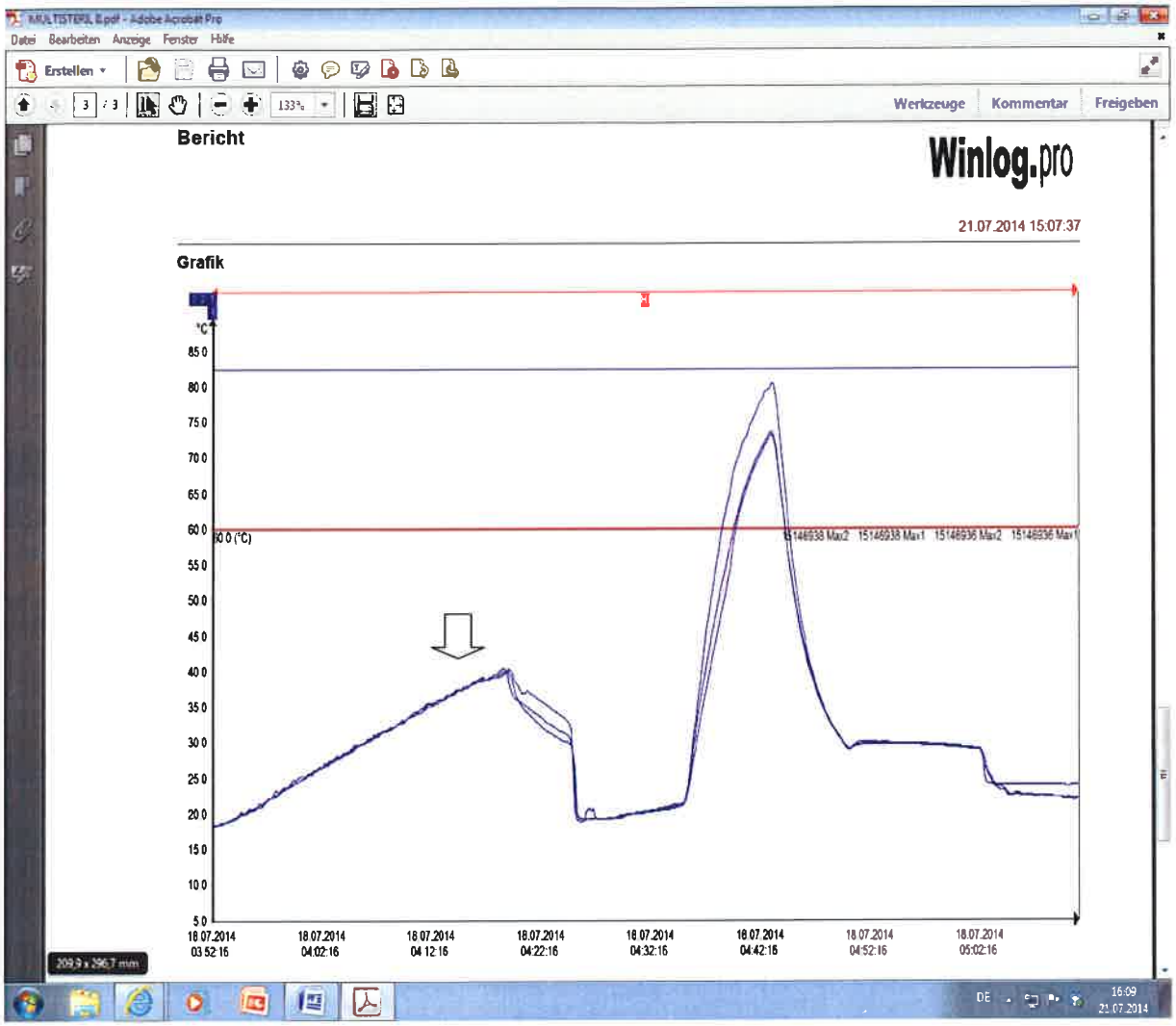

Figure 6: temperature profile of the liquid in the processing device (batch II)

The maximum process temperatures differ in the tested batches. In case of batch I, a maximum temperature of 37°C in the liquid is reached. The processing time (temperatures > 30°C) was approximately 14 minutes. In case of batch II, a maximum temperature of approximately 40°C in the liquid is reached. The processing time (temperatures > 30°C) was approximately 10 minutes.

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

3.3. Disinfection effect of the treatment method

Bioburden (number of colonies) before treatment (checks)

Number of colonies of the suspension	Enterococcus faecium in CFU / instrument
$1,0 \times 10^9$	$1,0 \times 10^8$

Bioburden (number of colonies) after treatment

Multisteril CD 0,5%	Enterococcus faecium in CFU / instrument
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0

Multisteril CD 1,0%	Enterococcus faecium in CFU / instrument
1	0
2	0
3	0
4	0
5	0
6	0
7	0
8	0
9	0
10	0

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

Water	Enterococcus faecium in CFU / instrument
1	> 1000
2	> 1000
3	> 1000
4	> 1000
5	> 1000
6	> 1000
7	> 1000
8	> 1000
9	> 1000
10	> 1000

3.4. Cleaning effect of the treatment method

Protein residue after treatment

Multisteril CD 0,5%	Protein residue (OPA) in µg / instrument
1	63
2	52
3	83
4	31
5	39
6	62
7	51
8	49
9	28
10	362

Multisteril CD 1,0%	Protein residue (OPA) in µg / instrument
1	36
2	54
3	64
4	35
5	26
6	41
7	81
8	52
9	53
10	27

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040

3.5. Assessment of the results

The tests performed could prove the effectiveness of the cleaning and disinfection of medical instruments in the MultiSteril processing device by TECNO-GAZ Industries in combination with Multisteril CD by ALPRO MEDICAL GMBH.

- The tests revealed a reduction of the bioburden (test germ: enterococcus faecium) by > 8 log levels (by means of cleaning and disinfection)

- The tests revealed a reduction of the contamination with coagulable blood to below the acceptance criteria of 100 µg / instrument in 19 of 20 instruments..

Dresden, 20 July 2014

Prof. Dr. med. E. Jacobs

Prof. Dr. Lutz Jatzwauk

Postanschrift:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fetscherstraße 74
01307 Dresden

Hausadresse:

Medizinische Fakultät Carl Gustav Carus
Technische Universität Dresden
Institut für Medizinische Mikrobiologie und Hygiene
Fiedlerstraße 42
01307 Dresden

Besuchsadresse:

Mtz
Fiedlerstraße 42
Raum: B.10.040