PERFORMANCE QUALIFICATION TEST ON ENDOSCOPE PRE-CLEANING DEVICE (EPD) ON OLYMPUS TJF-Q190V – MECHANICAL *Geobacillus stearothermophilus* (ATCC 7953) SPORE REMOVAL AND EVALUATION OF CLEANING EFFICACY

SPONSOR:

VAN VLIET MEDICAL SUPPLY BV.
WITGOUDWEG 51, 1362 JD,.
ALMERE,
THE NETHERLANDS.

TEST FACILITY:

EUROFINS BIOLAB SRL
VIA B. BUOZZI, 2
20055 VIMODRONE (MI)
ITALY

TEST ITEM: ENDOSCOPE PRE-CLEANING DEVICE (EPD)

QUOTATION N°: P6LTPH230585-04

Released by Study Director	***Signature & Date***
Silvia Carluccio	*Electronically signed on the last page of the document*

INDEX

COMPLIANCE WITH GOOD LABORATORY PRACTICE

I the undersigned declare that the study described in this report was conducted under my supervision and in compliance with the following standards of Good Laboratory Practice

- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring - OECD principles of Good Laboratory Practice (as revised in 1997) – Environment Directorate – Organization for Economic Co-Operation and Development, Paris 1998.

- Legislative decree n. 50, 02-Mar-2007, Enforcement of Community Directives 2004/9/CE and 2004/10/CE, concerning the inspection and verification of Good Laboratory Practice and the drawing of the legislative, regulatory and administrative dispositions relative to the application of Good Laboratory Practice rules, to the control of their application on the assays performed on the chemical substances (GU n.86, 13-Apr-2007).

- United States Food and Drug Administration, Title 21 Code of Federal Regulations Part 58, Federal Register 22-Dec-1978, and subsequent amendments

- GLP Certification (GLP Cert. 2024/3) released by the Italian Ministry of Health on 06-Feb-2024 authorizing Eurofins Biolab S.r.l. to perform analyses in compliance with the principles of good laboratory practices (http://www.eurofins.it).

- The experimental phase related to TOC determination has been performed By Ecotoxicology Internal Laboratory, under the responsibility of Cristina Giarei. The study phase was conducted under GLP certification.

There were no circumstances that may affect the quality or integrity of the study.

Study Director	***Signature & Date***
Silvia Carluccio	*Electronically signed on the last page of the document*

QUALITY ASSURANCE STATEMENT

The study was assessed for compliance with the approved Study Plan and the Standard Operating Procedures of Eurofins Biolab S.r.l.

The study and the Test Facility were periodically inspected by the Quality Assurance Unit, according to the corresponding SOPs. These inspections and audit were carried out by the Quality Assurance Unit, independent of staff involved in the study.

The undersigned hereby certifies the dates on which the inspections were carried out and reported to the Study Director and to Eurofins Biolab S.r.l. Management:

QA INSPECTION PHASE	DATE
Study Experimentation:	
- Process Based Audit	
Assay system preparation - Microorganisms	16/04/2025
Test material/item preparation	24-Jul-2024
Inoculum recovery/soiling (swabbing, flushing, soaking), neutralization and plating - Filtration	13-Dec-2024
Reading results - CFU (Bacteria and fungi)	14-Nov-2024
MD/carrier inoculum and drying	16/04/2025
Determination of organic/inorganic/dissolved carbon by TOC analyser	31-Dec-2024
Reading results - Visual observation	22-Oct-2024
Preparation of endoscope	14-Jan-2025
Residual protein with BCA kit or micro BCA kit	09-Apr-2024
- Study-Based Audit	//
Study Documentation:	
- Study Plan	17-Feb-2025
- Raw data	07-May-2025
- Final report	07-May-2025

The Final Report accurately reflects the raw data.

Quality Assurance Signature & Date

Electronically signed on the last page of the document

1. SUMMARY

The aim of the study was to evaluate the pre-cleaning effectiveness of Endoscope Pre-cleaning Device (EPD) on endoscope Olympus TJF-Q190V in terms of:

a) *Geobacillus stearothermophilus* (ATCC 7953) spore removal (according to EN ISO 15883-4:2019, B.3.4)

b) evaluation of cleaning efficacy (according to EN ISO 15883-4:2019, 6.11) through protein and total organic carbon (TOC) detection after endoscope contamination with ATS soil (as per EN15883-5)

A Graphic representation (not in scale, as declared by the Sponsor) of the endoscope Olympus TJF-Q190V is shown in Figure N.1:

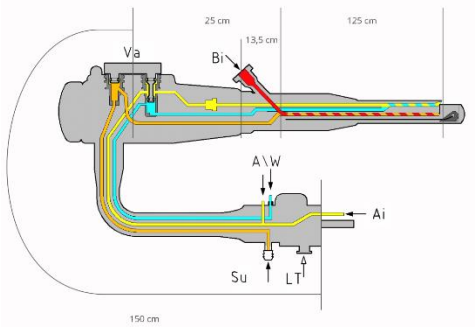


Figure N.1: endoscope Olympus TJF-Q190V

The length of each channel is declared by the Sponsor and are reported below:

Biopsy ∅ 4,2 mm x 138,5 cm

Suction ∅ 3,7 mm x 300 cm

Air	∅ 2,0 mm x 150 cm	+	∅ 1,0 mm x 150 cm
Water	∅ 2,0 mm x 150 cm	+	∅ 1,2 mm x 150 cm

Two screening studies has been performed on endoscope GF UCT180 to define the endoscope inoculation, recovery volume and to understand the trend of the pre-cleaning process with two Endoscope Pre-cleaning Device (EPD) parameters configuration (STULV24AA1951-3 and STULV24AA1951-4 respectively) for both spore removal and cleaning evaluation.

An additional screening step for cleaning evaluation has been performed on Olympus TJF-Q190V (STULV24AA1951-8).

Basing on the obtained results, the following Endoscope Pre-cleaning Device (EPD) configuration (see Figure N.2) was used in this GLP study:

Air pressure	3000	millibar
Air pulse	1750	milliseconds
Water pulse	1250	milliseconds
Water pressure	2000	millibar
Number of pulses	100	pulses

Figure N.2: Endoscope Pre-cleaning Device (EPD) parameters set up

Then, the following parameters for spray gun procedure was applied and another cleaning cycle wasperformed on the distal tip of the endoscope, according to Sponsor's Endoscope pre-cleaning device instructions for use (see Figure N3).

Air pressure	3000	millibar
Air pulse	750	milliseconds
Water pulse	750	milliseconds
Water pressure	2000	millibar
Number of pulses	40	pulses

Figure N.3: Endoscope Pre-cleaning Device (EPD) parameters Parameters of spray gun / distal end cleaning

The Endoscope Pre-cleaning Device's pre-cleaning procedure is a well-orchestrated series of steps, meticulously designed to guarantee traceability, safety, and unparalleled cleanliness of flexible endoscopes. The Endoscope Pre-cleaning Device was managed according to the Sponsor's Instructions for use. Set up, alarms and cycle parameters are under the responsibility of the Sponsor.

Three test replicas wasrun for both mechanical spore removal and cleaning evaluation.

<u>For physical spore removal</u>, the Endoscope Olympus TJF-Q190V wascontaminated with 10 ml of spore suspension soiling the suction and biopsy channels, as they are the worst case endoscope parts in terms of soil during real use condition. A schematic overview is shown in Figure N.4.

10 ml was flushed from the Suction port and the inoculum was pushed by flushing air until it was arrive at the level of the silicone tube connected to the biopsy channel opening; then, this point was clamped and air was flushed to also fill the entire portion of the biopsy/suction channel present in the insertion tube (which is the portion of the endoscope that actually enters the patient).

The inoculum was left internally for 1 hours at room temperature (drying time).

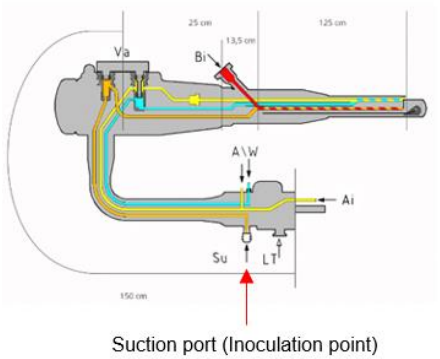


Figure N.4: Endoscope inoculation was performed from suction port.

At the end of drying time, the endoscope wasconnected to the Endoscope Pre-cleaning Device, according to the Sponsor's Instruction (See Attachment N.1) and the pre-cleaning cycle was start (for test), or the channels was flushed to recover the Positive control.

At the end of the procedure, an appropriate volume of peptone water plus tween 80, was flushed into each channel to quantify the spore that remain into the endoscope (see the experimental flow chart to volume details).

Basing on channel's diameters, similar channels was merged (relative area and maximum recovery volume calculated), so the recovery was performed considering the contribution of:

- Biopsy/suction channels (diameter >3mm)
- Air/Water channels (diameter of 2 mm)

Positive and negative controls and a validation of the filtration procedure wasperformed to assess the reliability of the method.

As one endoscope has been provided by the Sponsor, the experimental flow chart to perform the mechanical spore removal step is reported in table N.1 and it is followed for each replica:

Step number	Phase/control	Reactant
1	Preliminary Disinfection	PAA 10 minutes 2000 ppm by flushing
2	Pre-cleaning by EPD to remove disinfectant residues	//
3	Recovery of first Negative control **(Nc1)**	90 ml of peptone water plus tween 80 for each channel: suction, biopsy, air and water channels*
4	Microbial inoculation	*Geobacillus stearothermophilus (10 ml)*
5	Recovery of Positive control **(Pc)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy, air and water channels*
6	Disinfection step	PAA 10 minutes 2000 ppm by flushing
7	Pre-cleaning by EPD to remove disinfectant residues	//
8	Recovery of second Negative control **(Nc2)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy, air and water channels*
9	Microbial inoculation	*Geobacillus stearothermophilus (10 ml)*
10	Pre-cleaning by EPD to assess mechanical spore removal	//
11	Recovery of test **(test Na)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy, air and water channels*

* The recovery fluid of biopsy and suction was pooled together (180 ml)

** The recovery fluid of air and water was pooled together (180 ml)

Table N.1: flow chart to perform the mechanical spore removal evaluation

At the end of the described procedure, at least 10^5 endospores shall be recovered from the endoscope (cfu/channels >10^5). The contribution of physical removal is evaluated considering biopsy/suction channels. If the recovery is less than 10^5 cfu/channels, the Pc shall be considered as valid if the reduction obtained by difference between Pc and Na is less than 4 Log. In fact, in this case a sporicidal activity is not reached, but the mechanical removal can be considered effective.

For evaluation of cleaning efficacy: the Endoscope Olympus TJF-Q190V wascontaminated with 10 ml of ATS soil, as per EN15883-5. The inoculation procedure is the same described before for spore inoculation.

The difference is that the ATS soil, once flowed into the suction/biopsy channel, wasrecovered outside by flushing air. The soil volume recovered wasmeasured and the soil remained into the endoscope calculated by difference. Then, one hour of drying time was start.

At the end of drying time, the endoscope wasflushed with MilliQ water for Positive control device (Pc device) or it wasconnected to the Endoscope Pre-cleaning Device, according to the Sponsor's Instruction (See Attachment N.1) and the pre-cleaning cycle was start for test device (Na).

At the end of the procedure, an appropriate MilliQ water volume (it depends on the surface of the channels, i.e Biopsy/Suction: 400ml; Air/Water:300ml), wasflushed into the channels to quantify the protein and TOC content that remain into the endoscope.

Particularly.

- Biopsy/Suction→ about 357.96 cm^2 , 400 ml for a maximum recovery volume of 458 ml)
- Air/Water → about 292 cm^2 , 300 ml for a maximum recovery volume of 373 ml)

Similar channels was merged as previous described.

Positive and negative controls was performed to assess the reliability of the method and recovery efficiency.

As one endoscope has been provided by the Sponsor, the experimental flow chart to perform the cleaning step is reported in table N.2 and it is followed for each replica:

Step number	Phase/control	Reactant
1	Preliminary Cleaning + brushing	Neodisher Mediclean forte
2	Pre-cleaning by soaking and flushing to remove detergent residues	MilliQ water
3	Pre-cleaning by EPD (to subject the negative control device to the same cleaning procedure of test device)	//
4	Recovery of first Negative device control **(Nc1)**	MilliQ water
5	Soil inoculation	ATS soil (10 ml)
6	Recovery of Positive device control **(Pc)**	MilliQ water
7	Cleaning step + brushing	Neodisher Mediclean forte

8	Pre-cleaning by soaking and flushing to remove detergent residues	MilliQ water
9	Pre-cleaning by EPD (to subject the negative control device to the same cleaning procedure of test device)	//
10	Recovery of second Negative device control **(Nc2)**	MilliQ water
11	Soil inoculation	ATS soil (10 ml)
12	Pre-cleaning by EPD to assess cleaning effectiveness	//
13	Recovery of test **(test Na)**	MilliQ water
14	Negative sample control (Nc sample)	MilliQ water (This sample control is for quantifying the signal from the extraction vessel and fluid, without exposure of the fluid to either soil or device)
15	Positive sample control (Pc sample)	ATS soil (the volume to be used is the amount of soil that remain into the channels after positive device control)

Table N.2: flow chart for evaluation of cleaning efficacy

The evaluation of soiling residuals was done by means of:

- BCA kit (or micro-BCA kit) analysis
- TOC analysis

The amount of protein in the product extract is determined by comparing the extract measurement with the standard curve created by a regression line calculated from the measurement of the standard dilutions of known concentration. The standard and extract samples should be analyzed in the same way.

For both protein and TOC the evaluations was done on channel groups and not on endoscope in general.

A residual protein value ≤ 6.4 ug/cm^2 should be found on each group of channel after the pre-cleaning cycle to consider the process as effective.

Determination of organic carbon/inorganic carbon was done using an automatic TOC analyzer by Internal Eurofins Ecotoxicology Laboratory, under responsibility of Cristina Giarei.

A value ≤ 12 ug/cm^2 of TOC should be found on each group of channel after the pre-cleaning cycle to consider the process as effective.

Basing on the obtained results:

- More than 10^5 endospores cfu/channels have been recovered from Positive control (Pc, sum biopsy suction), for each test replica. The difference (in Log value) between Positive control (Pc) and test (Na) was less than 4 Log, for each test replica. This means that the EPD pre-cleaning cycle mechanically remove some spores and that the sporicidal activity is not reached, as required by the Standard ISO 15883-4.

- For each test replica, the endoscope resulted free of visible contamination (the recovery fluid was visually checked, as it is not possible to see the internal endoscope channels), except for positive control. The average determination of residual protein and TOC contents did not exceed the limit defined value of 6.4 µg/cm^2 and 12 µg/cm^2 respectively, according to AAMI ST 98:2022

2. INTRODUCTION

This study was carried out on behalf of the Sponsor on Endoscope Pre-cleaning Device (EPD)

This study was performed in the Test Facility Eurofins Biolab S.r.l. in Vimodrone (MI) – Via B. Buozzi n. 2 (Italy).

The information related to the analytical activity are here reported:

Test Method	*Start*	*End*	*Raw Data* *Review date*	*Researchers*
PERFORMANCE QUALIFICATION TEST ON ENDOSCOPE PRE-CLEANING DEVICE (EPD) ON OLYMPUS GF-UCT180 – MECHANICAL Geobacillus stearothermophilus (ATCC 7953) SPORE REMOVAL AND EVALUATION OF CLEANING EFFICACY	17/02/2025	09/04/2025	07/05/2025	S. Prodorutti M. Vescovi E. Motta I. Baccarini P. Ghislanzoni C. Possenti D. Facchetti C. Giarei

3. BIBLIOGRAPHY

- EN ISO 15883-4:2018 - Washer-disinfectors - Part 4: Requirements and tests for washer disinfectors employing chemical disinfection for thermolabile endoscopes.

- UNI EN ISO 15883-4:2019- Washer-disinfectors - Part 1: General requirements, terms and definitions and tests.

- UNI EN ISO 15883-5:2021 Washer-disinfectors - Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy

- UNI EN 13704:2018 - Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic, and institutional areas - Test method and requirements (phase 2/step 1).

- EN 17126:2018/ UNI EN 17126:2019- Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of sporicidal activity in the medical area - Test method and requirements (phase 2, step 1).

- AAMI ST 98: 2022 – Cleaning validation of health care products- requirements for development and validation of a cleaning process for medical devices.

- Pierce™ BCA Protein Assay Kit – Numbers 23225 Thermo Scientific

- Eurofins Internal Study STULV24AA1951-3

- Eurofins Internal Study STULV24AA1951-4

- Eurofins Internal Study STULV24AA1951-8

4. FILING

The Study Plan, the Final Report, Amendments (if present) and all raw data are filed in the archives of Eurofins Biolab S.r.l. for 10 years after the issuing of the Final Report.

At the end of the study the test item was kept until the end of the project, then it was returned to the Sponsor.

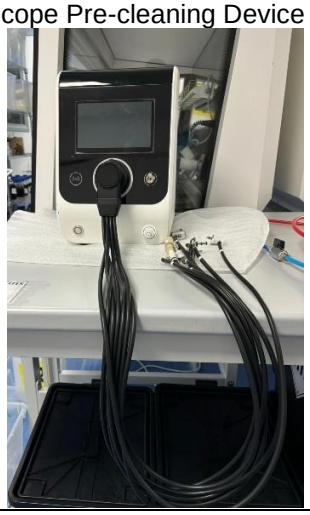
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the documents/products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

5. PROCEDURES

All procedures used during this study are recorded in the GLP Test Facility Eurofins Biolab S.r.l.

6. TEST ITEM AND ANALYZED SAMPLE

The test item consists of a Medical Device. One Endoscope Precleaning Device (EPD) has been provide by the Sponsor. It is designed to guarantee traceability, safety, and unparalleled cleanliness of flexible endoscopes.

Name	Endoscope Pre-cleaning Device (EPD) 
Code	Not Provided
Batch	Not Provided
Manufacturing date	Not provided
Stability	Sample is to be considered stable for the duration of the test
Received Date	22/10/2024
Incoming parcel number	IP-LV-2024296-AIK
Material aliquot number	LV-MAT-DR7G-24-297-0D83:a

6.1. TEST MATERIAL

The test material is the endoscope OLYMPUS TJF-Q190V. One endoscope has been provided by the Sponsor. It was connected to the EPD.

Name	OLYMPUS TJF-Q190V 
Code	Not Provided
Batch	Not Provided

Manufacturing date	Not provided
Stability	Sample is to be considered stable for the duration of the test
Received Date	19/08/2024
Incoming parcel number	IP-LV-2024232-ABL
Material aliquot number	LV-MAT-DR7G-24-253-0C48:a

The test item/material and the information concerning the test item/material were provided by the Sponsor. All data related to the test item/material are under the responsibility of the Sponsor and have not been verified by the test facility.

7. EXPERIMENTATION FOR PHYSICAL SPORE REMOVAL

7.1. ASSAY SYSTEM

Test strain
Geobacillus stearothermophilus ATCC 7953

Conservation of test strain
The bacterial spore suspension (ready to be used) is kept in a refrigerator at 5°C ± 3°C.

Preparation of the bacterial spore suspension
The spore suspension has been prepared outside of the study following the standard internal procedures, and according to EN17126:2018/ UNI EN 17126:2019.
It was diluted in water for injection (WFI) to a $1.0x10^7$ – $5.0x10^7$ cfu/ml, to obtain about 10^8 cfu/endoscope (10 ml of spore suspension was used to inoculate the endoscope).

Microorganism incubation condition
TSA plates with *Geobacillus stearothermophilus* was incubated for 48 hours at 57.5°C±2.5°C.

7.2. CULTURE MEDIA AND REAGENTS

Tryptone Soya Agar (TSA)
TSA plates
General diluent (Tryptone water)
WFI
Peptone water plus tween 80
Peracetic Acid (PAA)
RO water
MilliQ
Biosart Monitor filter 0.45 um

7.3. EQUIPMENT

Ordinary microbiological equipment and in particular:
Microbiological hood
Syringe
Vortexs
Silicon tubes*
Incubators (57.5°C ± 2.5 °C)
Chronometers
Micropipettes
Filtration system
Water bath
RO water system
MilliQ system

* silicon tubes of different size that were used for recovery have been cleaned after each step by soaking in Peracetic Acid (PAA)(38-40%) and rinsed in MilliQ.

7.4. EXCUTION OF THE ASSAY

Count of the bacterial spore suspension (N)
The bacterial spore suspension showing the defined concentration (between 1.0 $x10^7$ and $5.0x10^7$ cfu/ml) was diluted (up to 10^{-6}) with general diluent. 1 ml of 10^{-5}, 10^{-6} dilutions were pour plated in duplicate onto TSA. The number of colony forming units per ml (cfu/ml) of test suspension (**N**) was calculated.

Count of the bacterial spore validation suspension (Nv)
The bacterial spore suspension was diluted to a concentration between $3.0×10^2$ and $1.6×10^3$ cfu/ml (about one fourth (1+3) of the 10^{-4} dilution) in general diluent.
This suspension was further diluted with a decimal dilution with general diluent in order to perform a count by pour plate method. The highest number of cfu/plates was determined and **Nv** value then calculated.

Filtration control "B" – validation of the filtration procedure

0.1 ml of the validation suspension was taken in duplicate and transferred into a separate membrane filtration apparatus. The filtration procedure was performed immediately. 50 ml of rinsing liquid was added 3 times and filtered. Then, 50 ml of WFI was filtered and the membranes were transferred on TSA plates.
The number of colony-forming units per 0.1 ml of mixture was determined and **B** value calculated.

***Preparation of* endoscope Olympus TJF-Q190V and Negative control 1 (Nc1)**

The endoscope was preliminary cleaned by using Peracetic Acid (PAA)(38-40%) based disinfectant (provided by Eurofins). It was prepared at 2000 ppm final concentration by dilution in WFI. It was flushed (about 100 ml) into the endoscope by using the connectors system provided by the Sponsor. It was left inside for 10 minutes. Air was flushed into the channels by using a syringe to remove disinfectant. Then, the endoscope was connected to the EPD machine to remove any disinfectant residues. 90 ml of peptone water plus tween 80 for suction biopsy, air and water was flushed into the channels by using a syringe. The whole volume recovered was filtered. The cfu/channels was then calculated for each channel's group (biopsy/suction, air/water) (Nc1).

Step number	Phase/control	Reactant
1	Preliminary Disinfection	PAA 10 minutes 2000 ppm by flushing
2	Pre-cleaning by EPD to remove disinfectant residues	//
3	Recovery of first Negative control **(Nc1)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy*, air and water channels**

* The recovery fluid of biopsy and suction was pooled together (180 ml)

** The recovery fluid of air/water was pooled together (180 ml)

Endoscope Olympus TJF-Q190V inoculation

10 ml of spore suspensions ($1.0x10^7$ – $5.0x10^7$ cfu/ml) was flushed from the Suction port, and by flushing air (as shown in Figure N.4), the inoculum was pushed until it was arrive at the level of the silicone tube connected to the biopsy channel opening; then, this point was clamped and air was flushed to also fill the entire portion of the biopsy/suction channel present in the insertion tube (which is the portion of the endoscope that actually enters the patient). The inoculum was left dry for 1 hour at room temperature.

Step number	Phase/control	Reactant
4	Microbial inoculation	*Geobacillus stearothermophilus (10 ml)*

Recovery of Positive control (Pc)

At the end of drying time, 90 ml of peptone water plus tween 80 for suction biopsy, air and water channels was flushed into the channels by using a syringe. The volume recovered was diluted until 10^{-5} and 1 ml of each dilution pour plated in duplicate onto TSA. The remain volume was filtered. The cfu/channels was then calculated (**Pc**).

Step number	Phase/control	Reactant
5	Recovery of Positive control **(Pc)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy*, air and water channels**

* The recovery fluid of biopsy and suction was pooled together (180 ml)

** The recovery fluid of air/water was pooled together (180 ml)

Preparation of **endoscope Olympus TJF-Q190V and Negative control 2 (Nc2)**

The endoscope was treated with PAA 2000 ppm for 10 minutes as described for Negative control 1. Then, the EPD cycle was start to remove disinfectant residues. 90 ml of peptone water plus tween 80 for suction biopsy, air and water channels was flushed into the channels by using a syringe. The whole volume recovered was filtered. The cfu/channels was then calculated (Nc1).The cfu/channels was calculated (**Nc2**).

Step number	Phase/control	Reactant
6	Disinfection step	PAA 10 minutes 2000 ppm by flushing
7	Pre-cleaning by EPD to remove disinfectant residues	//
8	Recovery of second Negative control **(Nc2)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy*, air and water channels**

* The recovery fluid of biopsy and suction was pooled together (180 ml)

** The recovery fluid of air/water was pooled together (180 ml)

Endoscope Olympus TJF-Q190V inoculation

The endoscope inoculation was performed, as previously reported.

Step number	Phase/control	Reactant
9	Microbial inoculation	*Geobacillus stearothermophilus (10 ml)*

Assessment of physical spore removal (Na)

At the end of drying time, EPD cycle was started. At the end of the process, 90 ml of peptone water plus tween 80 for suction biopsy, air and water channels was flushed into the channels by using a syringe. The volume recovered was diluted until 10^{-5} and 1 ml of each dilution pour plated in duplicate onto TSA. The remain volume was filtered. The cfu/channels was then calculated (**Na**)

Step number	Phase/control	Reactant
10	Pre-cleaning by EPD to assess mechanical spore removal	//
11	Recovery of test **(test Na)**	90 ml of peptone water plus tween 80 for each channel: suction biopsy*, air and water channels**

* The recovery fluid of biopsy and suction was pooled together (180 ml)

** The recovery fluid of air/water was pooled together (180 ml)

7.5. CALCULATION

The viable count (Vc) values was determined as follows: the usual limits for counting bacteria on agar plates are between 15 and 300. A deviation of 10 % is accepted, so the limits are 14 and 330. On membranes, the usual upper limits are different: 150, therefore with the 10 % deviation, the limit is 165. However, the lower countable limit in test Na was set as 1 colony.
When rounding ciphers: if the last cipher is higher than or equal to '5' the previous cipher is increased of a unit; if the last cipher is lower than 5 the previous cipher remains unchanged; proceed this way until reaching two significant ciphers.

Test suspensions (N)

The number of cfu/ml of the test suspensions was calculated by applying the following formula:

$$N = \left[\frac{c}{(n_1 + 0.1n_2)\,\mathrm{d}} \right]$$

Where:

c = is the sum of *Vc* values taken into account
n_1 = is the number of *Vc* values taken into account in the lower dilution
n_2 = is the number of *Vc* values taken into account in the higher dilution
d = dilution factor corresponding to the lower dilution

Validation suspension (Nv)

The number of microorganisms per ml in the validation suspension (**Nv**) was determined using the following formula:

$$Nv = 10\frac{c}{n}$$

where:

c = is the sum of *Vc* values taken into account
n = is the number of *Vc* values taken into account
10 = dilution factor

Verification of the filtration procedure (B)

The number of cfu/ml in the validation control was calculated as following:

$$B = \frac{c}{n}$$

Where:

c = is the sum of *Vc* values taken into account
n = is the number of *Vc* values taken into account

Positive control (Pc) and Test (Na)

The number of cfu/channels of the test suspension in positive control and in test was calculated by applying the following formula:

$$\mathrm{Pc,\,Na} = \frac{c}{n} \times d \times V$$

Where:

c = is the sum of *Vc* values taken into account
n = is the number of *Vc* values taken into account
d = is the dilution factor
V = is the recovery volume/channels

This value was then converted in Log_{10}.

Negative controls (Nc1 and Nc2)
The number of cfu/channels of the test suspension in Negative controls was calculated by applying the following formula:

$$Nc1, Nc2 = c$$

Where:
c = is the sum of *Vc* values taken into account

This value was then converted in Log_{10}.

7.6. VALIDITY CRITERIA
N: shall be between 1.0×10^7 and 5.0×10^7 cfu/ml
Nv: shall be between 3.0×10^2 and 1.6×10^3 cfu/ml
B: is equal to or greater than 0.05 × Nv
Pc: endospores shall be $\geq10^5$ (cfu/channels) (for biopsy/suction)
Na: endospores shall be $>10^5$ (cfu/channels) (for biopsy/suction)
Nc1 and Nc2: no growth should be observed

Reduction factor for physical removal
The reduction in viable counts attributed to the EPD cycle was calculated by difference between the microbial recovery on positive control (Pc) and the microbial recovery in test (Na).
The reduction factor R was expressed as logarithm and calculated for group of channels and test replica, according to the following formula:

$$R = Log\ Pc - Log\ Na$$

7.7. INTERPRETATION OF RESULTS
At the end of the EPD pre-cleaning cycle, at least 10^5 endospores cfu/channels shall be recovered.
However, when the recovery is less than 10^5 cfu/channels, the Pc shall be considered as valid if the reduction obtained by difference between Pc and Na is less than 4 Log. In fact, in this case a sporicidal activity is not reached, while the mechanical removal can be considered achieved.

8. EXPERIMENTATION (FOR CLEANING EFFECTIVNESS)

8.1. REAGENTS AND MATERIALS
ATS soil
BCA kit: Protein assay Kit
MilliQ water (purified sterile deionized water)
NEODISHER MEDICLEAN FORTE
RO water
My wipe Polycell

8.2. EQUIPMENT
Usual common laboratory equipment and in particular:
Syringes
Silicon Tubes*
Chronometers
Micropipettess
Carbon analyser
Spectrophotometer
Mobile phone
Thermostatic water bath
RO water system
MilliQ water system

*silicon tubes of different size that have been used for recovery were cleaned after each step by soaking in Neodisher mediclen detergent and rinsed in MilliQ

8.3. EXPERIMENTAL DESIGN

Soil preparation

ATS soil was prepared following ISO 15883-5, with exception of heat inactivated bovine calf serum that was replaced with heat inactivated fetal bovine serum.

***Preparation of* endoscope Olympus TJF-Q190V and Negative control device 1 (Nc device1)**

The endoscope was preliminary cleaned by using Neodisher Mediclean Forte detergent (provided by Eurofins). It was prepared at 10 ml/l final concentration by dilution in MilliQ water. The endoscope was soaked 10 minutes in detergent (about 10 l). Brushes was used to mechanically clean the biopsy/suction endoscope internal channels. The detergent was flushed (about 100 ml) into the endoscope by using the connectors system provided by the Sponsor and air was flushed into the channels by using a syringe to remove detergent.

Then, the endoscope was completely soaked in MilliQ water (about 10L) and flushing (about 100 ml) by using a syringe was applied to remove detergent residues. No soil was applied. After the soaking steps, the endoscope was dried externally by using wipes and internally by air flow inside the channels.

EPD cycle was started. At the end of the process, an appropriate MilliQ water volume, was flushed into the channels by using silicon tubes of different size, that after each different step, was cleaned by soaking in Neodisher mediclean detergent and rinsed in MilliQ. Protein content and TOC content was then evaluated (**Nc device 1**)

Step number	Phase/control	Reactant
1	Preliminary Cleaning + brushing	Neodisher Mediclean forte
2	Pre-cleaning by soaking and flushing to remove detergent residues	MilliQ water
3	Pre-cleaning by EPD (to subject the negative control device to the same cleaning procedure of test device)	//
4	Recovery of first Negative control device **(Nc device 1)**	MilliQ water (Biopsy/Suction: 400ml; Air/Water: 300ml)

Endoscope Olympus TJF-Q190V soiling

The soiling procedure is the same described before for spore inoculation.

The difference is that 10 ml of ATS soil, once flowed into the suction/biopsy channel, was recovered outside by flushing air. The soil volume recovered was measured and the soil remained into the endoscope calculated by difference. Then, 1 hour of drying time was started.

Step number	Phase/control	Reactant
5	Soil inoculation	ATS soil (10 ml)

Recovery of Positive control device (Pc)

At the end of drying time, 400 ml of MilliQ water for biopsy/suction, 300 ml for air/water channels was

flushed into the channels by using a syringe. The volume recovered was analyzed for residual protein and TOC content (Pc device).

Step number	Phase/control	Reactant
6	Recovery of Positive control device **(Pc device)**	MilliQ water (Biopsy/Suction: 400ml; Air/Water:300ml)

Preparation of **endoscope Olympus TJF-Q190V and Negative control device 2 (Nc device2)**

The endoscope was preliminary cleaned by using Neodisher Mediclean Forte detergent as previous described. Brushes were used to mechanically clean the biopsy/suction endoscope internal channels.

Then, the endoscope was completely soaked in MilliQ water as previous described. No soil was applied. After the soaking steps, the endoscope was dried externally by using wipes and internally by air flow inside the channels.

EPD cycle was started. At the end of the process, an appropriate MilliQ water volume, was flushed into the channels by using silicon tubes of different size, that after each different step, was cleaned by soaking in Neodisher mediclen detergent and rinsed in MilliQ. Protein content and TOC content was then evaluated (**Nc device 2**)

Step number	Phase/control	Reactant
7	Cleaning step + brushing	Neodisher Mediclean forte
8	Pre-cleaning by soaking and flushing to remove detergent residues	MilliQ water
9	Pre-cleaning by EPD (to subject the negative control device to the same cleaning procedure of test device)	//
10	Recovery of second Negative device control **(Nc device2)**	MilliQ water (Biopsy/Suction: 400ml; Air/Water:300ml)

Endoscope Olympus TJF-Q190V soiling

The endoscope soiling was performed, as previously reported.

Step number	Phase/control	Reactant
11	Soil inoculation	ATS soil (10 ml)

Assessment of cleaning efficacy (Na)

At the end of drying time, EPD cycle was started. At the end of the process, 400 ml of MilliQ water for biopsy/suction and 300 ml for air/water channels was flushed into the channels by using a syringe. The volume recovered was analyzed for residual protein and TOC content (Na).

Step number	Phase/control	Reactant
12	Pre-cleaning by EPD to assess cleaning effectiveness	//

13	Recovery of test **(test Na)**	MilliQ water (Biopsy/Suction: 400ml; Air/Water:300ml)

Negative sample control (Nc sample)

The protein and TOC content was measured for the recovery liquid (MilliQ water).

This sample control was performed for quantifying the signal from the extraction vessel and fluid, without exposure of the fluid to either soil or device.

Step number	Phase/control	Reactant
14	Negative sample control (Nc sample)	MilliQ water

Positive sample control (Pc sample)

The protein and TOC content was measured for the ATS soil. The volume of soil that remain into Biopsy/suction channels after positive control device was used for calculation. This amount of soil was added to 400 ml of MilliQ water, that is the recovery volume for biopsy/suction channels.

Step number	Phase/control	Reactant
15	Positive sample control (Pc sample)	ATS soil (the volume to be used is the amount of soil that remain into the channels after positive device control)

8.4. CALCULATION

Recovery

All endoscope channels (groups of channels as previous described) was recovered in MilliQ water (extraction fluid) according to a validated method for extraction, that was performed to demonstrate the recovery efficiency.

Particularly, an appropriate volume of MilliQ water (Biopsy/Suction: 400ml; Air/Water:300ml) was flushed into the channels.

The recovery efficiency for biopsy/suction channels should be as high as practical, but generally should be greater than 70%.

Evaluation of residual soil

For each groups of channel and each control/test, two aliquots of the extraction fluid were taken: an aliquot was analyzed in TOC analyzer and an aliquot was analyzed to determine the residual protein by BCA kit “enhanced protocol”.

Determination of residual protein (BCA kit)

The accurate quantification of residual protein is dependent upon the preparation of the protein to be used as the reference standard for the method. The standard protein used for calibration is bovine serum albumin (BSA, fraction V). The amount of protein in the product extract was determined by comparing the extract measurement with the standard curve created by a regression line calculated from the measurement of the standard dilutions of known concentration. The standard and extract samples should be analyzed in the same way. Residual protein determination was performed following the “enhanced protocol” of BCA kit. Limit of detection of the enhanced protocol is 5 µg/mL.
Residual protein was analyzed with a spectrophotometer at a wavelength of 562 nm.
A calibration curve was performed in MilliQ at a wavelength of 562 nm.

Negative control was subjected to the same treatment of the test device and tested for residual proteins, in order to determine any possible interference between tested products and the method used for proteins measurement.
Results obtained was noted (Nc device)
Protein concentration remained on the surface of the channels after each procedure was calculated as indicated below:

$$\text{Na Protein } \mu g/cm^2 = [\text{Test Na } (\mu g/cm^2)] - [\text{Nc device 2 } (\mu g/cm^2)]$$

$$\text{Pc Protein } \mu g/cm^2 = [\text{Pc device } (\mu g/cm^2)] - [\text{Nc device 1 } (\mu g/cm^2)]$$

Determination of total organic carbon (TOC)
Determination of organic carbon/inorganic carbon was done using an automatic TOC analyzer.
It was used a TOC with a high sensitivity based on the combustion catalytic oxidation method (680°C) in which the carbon dioxide generated by oxidation is detected using an infrared gas analyzer (NDIR).
Samples, properly diluted with deionized water with low TOC contents, were analyzed by means of an integrated sampling system directly from vials with 40 mL volume.
Limit of detection of the TOC is 50 µg/L.
Negative control was subjected to the same treatment of the test device and tested for TOC in order to determine any possible interference between tested products and the method used for TOC detection.
Results obtained have been noted [TOC Nc device]
From that time, TOC concentration remained on the surface of the test tubes after each procedure ([TOC]) has been calculated as indicated below:

$$\text{Na TOC } \mu g/cm^2 = [\text{TOC Na}] - [\text{TOC. Nc device 2}]$$
$$\text{Pc TOC } \mu g/cm^2 = [\text{TOC Pc device}] - [\text{TOC. Nc device 1}]$$

8.5. VALIDITY CRITERIA
- After EPD cycle, the endoscope shall be free of visible contamination (the recovery fluid was visually checked, as it is not possible to see the internal endoscope channels).
The positive, the negative controls and the test item were inspected visually to identify appearing debris.
-Limit value of residual protein content: ≤6.4 µg/cm^2
-Limit value TOC: ≤12 µg/cm^2

9. RESULTS
Biopsy/suction channels results are presented in the results section as these are the channels that have an impact on the interpretation of the study results.

Results from air/water channels are collected in the raw data and are not shown in this report.

9.1. PHYSICAL SPORE REMOVAL (REPLICA 1, 2 AND 3)
Preliminary assay
The validation tests comply with the validity criteria. The specific values are shown in Addendum N.2,3,4.

Assay
The main results have been summarized in the tables below.

			Log Pc-Log Na (Log cfu/channels) Replica 1
Pc (sum biopsy suction) cfu/channel	**Log Pc Sum**	**Valid/not Valid**	**Biopsy+suction**
5.8E+07	7.76	valid	**3.91**

			Log Pc-Log Na (Log cfu/channels) Replica 2
Pc (sum biopsy suction) cfu/channel	**Log Pc Sum**	**Valid/not Valid**	**Biopsy+suction**
1.2E+08	8.08	valid	**3.87**

			Log Pc-Log Na (Log cfu/channels) Replica 3
Pc (sum biopsy suction) cfu/channel	**Log Pc Sum**	**Valid/not Valid**	**Biopsy+suction**
1.8E+08	8.26	valid	**3.87**

9.2. CLEANING EFFECTIVNESS REPLICA 1

9.2.1. Visual Evaluation results

The visual evaluation results after cleaning procedure are reported in table below.

VISUAL EVALUATION RESULTS		
SAMPLE	**Evaluation**	
Positive DEVICE control	☑ Debris	☐ No debris
Negative DEVICE control 1	☐ Debris	☑ No debris
Negative DEVICE control 2	☐ Debris	☑ No debris
Test biopsy/suction channels	☐ Debris	☑ No debris
*the visual evaluation was performed on extraction fluid		

9.2.2. Residual protein by BCA kit

Recovery Rate: 114.5%

Correction Factor:1.00

The calculated correction factor has been considered for the determination of the protein contents of the controls and the cleaned devices.

Calibration curve

The graph below shows the spectrophotometric calibration curve (Figure N. 4)

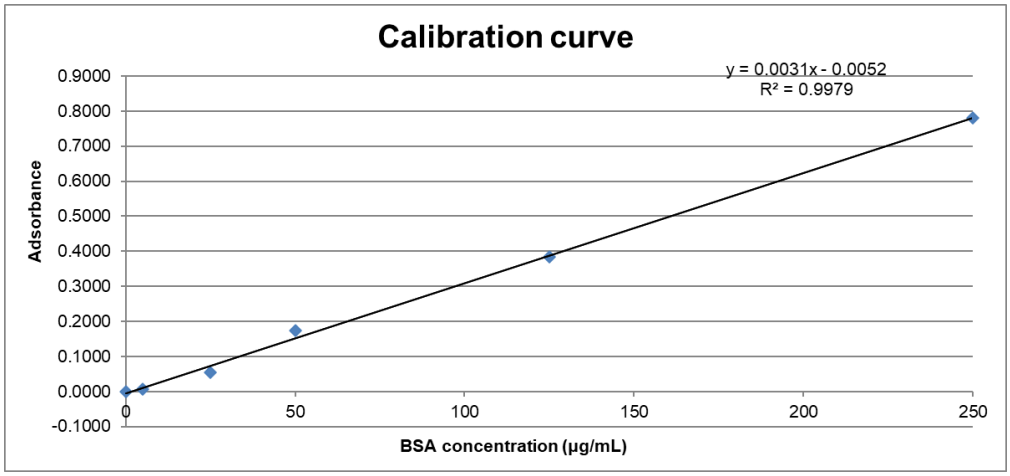


Figure N.4: calibration curve Replica 1

The limit of detection is 5 µg/ml. In case of values below the analytical sensitivity, the mentioned analytical sensitivity limit is used for calculation.

RESULTS AND COMPLIANCE		
	Average Residual proteins concentration (µg/ml)	**Residual proteins value - (µg/channel)***
Positive DEVICE control	396.0	158397.0
Negative DEVICE control 1	0.0	Not Applicable
Negative DEVICE control 2	0.5	Not Applicable
Positive SAMPLE control	346.4	Not Applicable
Negative SAMPLE control	0.6	Not Applicable
Test biopsy/suction channels	0.0	0.0

* Residual proteins value Test Item – residual protein Negative DEVICE control x CF

CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE					
SAMPLE	Residual proteins (µg/cm²)	Limit Residual proteins (µg/cm²)		Results of residual proteins detected using *enhanced protocol* (µg/cm²)	PASS/FAIL
Test biopsy/suction channels	0.0	<	5.6	< 5.6	PASS

Residual protein limit value related to the surface soiled and extraction volume used. Limit value from guideline (µg/cm²) is ≤6.4.

9.2.3. TOC results

Recovery Rate: 104.3%

Correction Factor:1.00

The calculated correction factor has been considered for the determination of the TOC contents of the controls and the cleaned devices.

SAMPLE	Mean NPOC value (mg/L)
Positive DEVICE control	151.300
Negative DEVICE control 1	0.523
Negative DEVICE control 2	0.515
Positive SAMPLE control	145.700
Negative SAMPLE control	1.154
Test biopsy/suction channels	0.520

RESULTS AND COMPLIANCE		
	Residual NPOC (µg/channels)	**Residual NPOC value - (µg/channels)***
Positive DEVICE control	60520.0	60311.0
Negative DEVICE control 1	209.0	Not Applicable
Negative DEVICE control 2	205.9	Not Applicable
Positive SAMPLE control	58280.0	Not Applicable
Negative SAMPLE control	461.7	Not Applicable
Test biopsy/suction channels	207.9	0.0

* Residual NPOC value Test Item – residual protein Negative DEVICE control x CF CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE					
SAMPLE	Residual TOC concentration (µg/cm²)	Limit Residual TOC concentration (µg/cm²)		Results of residual TOC concentration (µg/cm²)	PASS/FAIL
Test biopsy/suction channels	0.0	≤	12.0	< 12.0	PASS

9.3. CLEANING EFFECTIVNESS REPLICA 2

9.3.1. Visual Evaluation results

The visual evaluation results after cleaning procedure are reported in table below.

VISUAL EVALUATION RESULTS		
SAMPLE	**Evaluation**	
Positive DEVICE control	☑ Debris	☐ No debris
Negative DEVICE control 1	☐ Debris	☑ No debris
Negative DEVICE control 2	☐ Debris	☑ No debris
Test biopsy/suction channels	☐ Debris	☑ No debris
*the visual evaluation was performed on extraction fluid		

9.3.2. Residual protein by BCA kit
Recovery Rate: 95.1%
Correction Factor:1.05

The calculated correction factor has been considered for the determination of the protein contents of the controls and the cleaned devices.

Calibration curve

The graph below shows the spectrophotometric calibration curve (Figure N. 5)

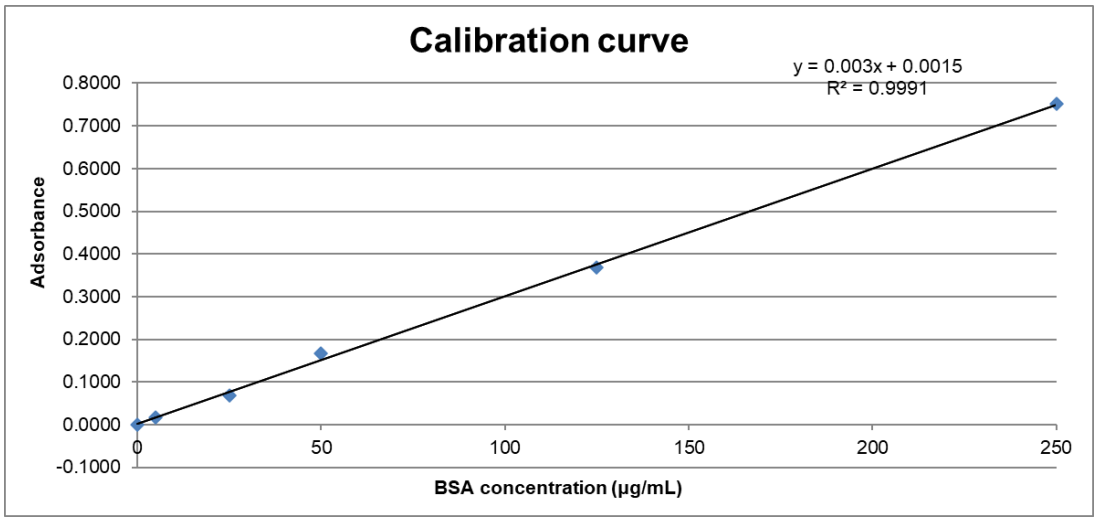


Figure N.5: calibration curve Replica 2

The limit of detection is 5 µg/ml. In case of values below the analytical sensitivity, the mentioned analytical sensitivity limit is used for calculation.

RESULTS AND COMPLIANCE		
	Average Residual proteins concentration (µg/ml)	**Residual proteins value - (µg/channel)***
Positive DEVICE control	420.2	174702.7
Negative DEVICE control 1	4.9	Not Applicable
Negative DEVICE control 2	5.1	Not Applicable
Positive SAMPLE control	443.6	Not Applicable
Negative SAMPLE control	6.9	Not Applicable
Test biopsy/suction channels	2.9	0.0

* Residual proteins value Test Item – residual protein Negative DEVICE control x CF CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE					
SAMPLE	Residual proteins (µg/cm²)	Limit Residual proteins (µg/cm²)		Results of residual proteins detected using *enhanced protocol* (µg/cm²)	PASS/FAIL
Test biopsy/suction channels	0.0	<	5.6	< 5.6	PASS

residual protein limit value related to the surface soiled and extraction volume used. Limit value from guideline (µg/cm^2) is ≤6.4.

9.3.3. TOC results
Recovery Rate: 118.6%

Correction Factor:1.00

The calculated correction factor has been considered for the determination of the TOC contents of the controls and the cleaned devices.

SAMPLE	Mean NPOC value (mg/L)
Positive DEVICE control	163.067
Negative DEVICE control 1	0.480
Negative DEVICE control 2	0.537
Positive SAMPLE control	137.533
Negative SAMPLE control	0.439
Test biopsy/suction channels	0.500

RESULTS AND COMPLIANCE		
	Residual NPOC (µg/channels)	**Residual NPOC value - (µg/channels)***
Positive DEVICE control	65226.7	65034.5
Negative DEVICE control 1	192.2	Not Applicable
Negative DEVICE control 2	214.7	Not Applicable
Positive SAMPLE control	55013.3	Not Applicable
Negative SAMPLE control	175.7	Not Applicable
Test biopsy/suction channels	200.1	8.0

* Residual NPOC value Test Item – residual protein Negative DEVICE control x CF CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE					
SAMPLE	**Residual TOC concentration (µg/cm^2)**	**Limit Residual TOC concentration (µg/cm^2)**		**Results of residual TOC concentration (µg/cm^2)**	**PASS/FAIL**
Test biopsy/suction channels	0.0	≤	12.0	≤ 12.0	**PASS**

9.4. CLEANING EFFECTIVNESS REPLICA 3

9.4.1. Visual Evaluation results
The visual evaluation results after cleaning procedure are reported in table below.

VISUAL EVALUATION RESULTS		
SAMPLE	**Evaluation**	
Positive DEVICE control	☑ Debris	☐ No debris
Negative DEVICE control 1	☐ Debris	☑ No debris
Negative DEVICE control 2	☐ Debris	☑ No debris
Test biopsy/suction channels	☐ Debris	☑ No debris
*the visual evaluation was performed on extraction fluid		

9.4.2. Residual protein by BCA kit
Recovery Rate: 123.5%
Correction Factor:1.00
The calculated correction factor has been considered for the determination of the protein contents of the controls and the cleaned devices.

Calibration curve

The graph below shows the spectrophotometric calibration curve (Figure N.6)

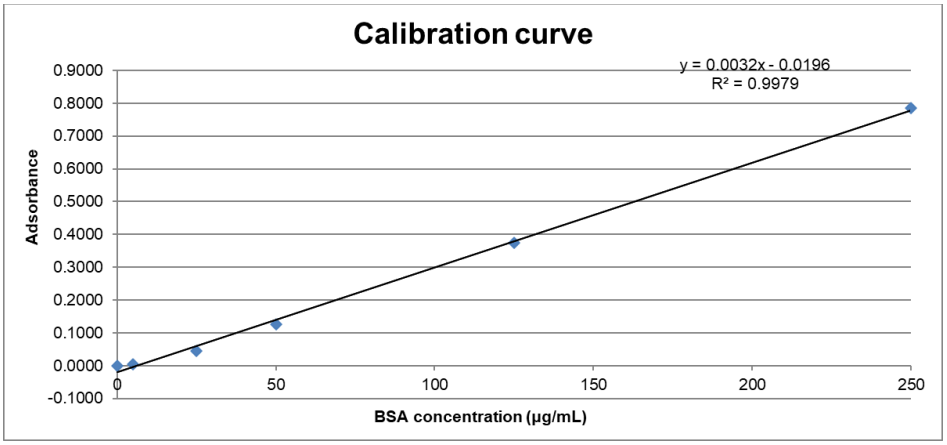


Figure N.6: calibration curve Replica 3

The limit of detection is 5 µg/ml. In case of values below the analytical sensitivity, the mentioned analytical sensitivity limit is used for calculation.

RESULTS AND COMPLIANCE		
	Average Residual proteins concentration (µg/ml)	**Residual proteins value - (µg/channels)***
Positive DEVICE control	473.9	187827.3
Negative DEVICE control 1	4.4	Not Applicable
Negative DEVICE control 2	5.1	Not Applicable
Positive SAMPLE control	384.9	Not Applicable
Negative SAMPLE control	4.6	Not Applicable

Test biopsy/suction channels	5.7	526.8

* Residual proteins value Test Item – residual protein Negative DEVICE control x CF CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE				
SAMPLE	Residual proteins (µg/cm²)	Limit Residual proteins (µg/cm²)	Results of residual proteins detected using *enhanced protocol* (µg/cm²)	PASS/FAIL
Test biopsy/suction channels	1.5	< 5.6	< 5.6	PASS

residual protein limit value related to the surface soiled and extraction volume used. Limit value from guideline (µg/cm²) is ≤6.4.

9.4.3. TOC results
Recovery Rate: 105.8%

Correction Factor:1.00

The calculated correction factor has been considered for the determination of the TOC contents of the controls and the cleaned devices.

SAMPLE	Mean NPOC value (mg/L)
Positive DEVICE control	150.567
Negative DEVICE control 1	0.551
Negative DEVICE control 2	0.576
Positive SAMPLE control	142.267
Negative SAMPLE control	0.479
Test biopsy/suction channels	0.544

RESULTS AND COMPLIANCE		
	Residual NPOC (µg/channels)	**Residual NPOC value - (µg/channels)***
Positive DEVICE control	60226.7	60006.3
Negative DEVICE control 1	220.4	Not Applicable
Negative DEVICE control 2	230.5	Not Applicable
Positive SAMPLE control	56906.7	Not Applicable
Negative SAMPLE control	191.6	Not Applicable
Test biopsy/suction channels	217.4	0.0

* Residual NPOC value Test Item – residual protein Negative DEVICE control x CF CF = correction factor

Results related to biopsy/suction channels are shown below.

RESULTS AND COMPLIANCE					
SAMPLE	Residual TOC concentration (µg/cm²)	Limit Residual TOC concentration (µg/cm²)		Results of residual TOC concentration (µg/cm²)	PASS/FAIL
Test biopsy/suction channels	0.0	≤	12.0	≤ 12.0	PASS

10. DEVIATION

The deviation EXC- LV25AA6829 was recorded to notify that the PAA was used after the expiry date in the third test replica for spore removal, according to the Sponsor.

11. CONCLUSIONS

On the basis of the results, the test item "Endoscope Pre-cleaning Device (EPD)" in parameters configuration reported in Figure N.2, **MEETS THE REQUIREMENT** for both spore removal and cleaning efficacy.

Particularly, after the EPD cycle selected:

- More than 10^5 endospores cfu/channels have been recovered from Positive control (Pc, sum biopsy suction), for each test replica. The difference (in Log value) between Positive control (Pc) and test (Na) was less than 4 Log, for each test replica. This means that the EPD pre-cleaning cycle mechanically remove some spores and that the sporicidal activity is not reached, as required by the Standard ISO 15883-4.
- For each test replica, the endoscope resulted free of visible contamination (the recovery fluid was visually checked, as it is not possible to see the internal endoscope channels), except for positive control. The average determination of residual protein and TOC contents did not exceed the limit defined value of 6.4 µg/cm² and 12 µg/cm² respectively, according to AAMI ST 98:2022

12. ADDENDA

ADDENDUM	TITLE	NUMBER OF PAGES
N.1	ENDOSCOPE PRE-CLEANING DEVICE – INSTRUCTIONS FOR USE - DEMO	12
N.2	RAW DATA EXPERIMENTATION SPORE REMUVAL REPLICA 1	2
N.3	RAW DATA EXPERIMENTATION SPORE REMUVAL REPLICA 2	2
N.4	RAW DATA EXPERIMENTATION SPORE REMUVAL REPLICA 3	2

13. ATTACHMENT N. 1

SIGNATURES

Electronic signatures by Eurofins Biolab srl reported at the bottom of the document certify its approval and represent the confirmation of its content.

The software eLIMS-BPT is designed and validated following 21 CFR Part 11 requirements. The electronically signed records include controls to confirm the authenticity of the signature on signed documents. Audit trails are captured to identify signing events and changes to electronic signature records.

Reviewed and electronically signed for Study Quality Assurance Approval by
Noemi Isacchi, Employee
for Eurofins Biolab Srl, on 08-May-2025 09:27:32 UTC+02:00
Reviewed and electronically signed for GLP Study Director Approval by
Silvia Carluccio, Employee
for Eurofins Biolab Srl, on 08-May-2025 10:18:00 UTC+02:00

STULV24AA1951-6 GLP

ENDOSCOPE PRE-CLEANING DEVICE INSTRUCTIONS FOR USE - DEMO

VAN VLIET HEALTHCARE
MEDICAL

VAN VLIET HEALTHCARE MEDICAL

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author	**Document ID**	**Revision**
Bo Koperdraat	DOC-XXXX	0

ENDOSCOPE PRE-CLEANING DEVICE – INSTRUCTIONS FOR USE - DEMO 2

VAN VLIET HEALTHCARE MEDICAL

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author	**Document ID**	**Revision**
Bo Koperdraat	DOC-XXXX	0

ENDOSCOPE PRE-CLEANING DEVICE – INSTRUCTIONS FOR USE - DEMO

In this document the working principle of comprex, the process of the endoscope pre-cleaning device, and the instructions for use of the demo unit will be discussed.

1. Comprex™ working principle

In contrast to conventional water flushing, comprex uses targeted pulses of compressed air and water. The process therefore works purely mechanically without aggressive chemicals and still achieves the desired cleaning effect for all types of pipelines.

Once the adapter is connected to the endoscope and the process is started, compressed air and water is introduced into the channels in a controlled and pulsed manner. Inside the pipeline – with the water remaining there – packets of air and water blocks are created, which flow through the pipeline section at high speed.

These packages generate enormous turbulence with strong shear and drag forces that mobilize and reliably discharge the deposits on the pipe walls.

The comprex Medical story started in 2005 with an initial contact with the Institute for Hygiene and Public Health at the University of Bonn, under the leadership of Prof. Dr. med. Martin Exner, who confirmed to us the cleaning performance of comprex on his biofilm silicone hose model in an expert opinion.

This resulted in the participation in a first BMBF research project on biofilms in domestic installations under the leadership of Prof. Dr. Hans-Curt Flemming of the University of Duisburg-Essen, Prof. Dr. Exner and Dr. Jürgen Gebel, head of the laboratory at the institute of Prof. Exner. Dr. Gebel was the contact person regarding studies and research from the beginning.

This was followed by further research projects and the development of additional test equipment used at the Bonn institute for cleaning endoscope dummies. Two dissertations from 2014 and 2018, supervised by Prof. Dr. Exner, have again confirmed the extraordinary cleaning performance of comprex. As a budding physicist, Paulina Lämmer was already working on the project Brushless Cleaning of PTFE Test Pieces as a Surrogate of Endoscope Channels back then.

This is the nucleus of comprex Medical and the Endoscope Pre-cleaning Device. The extraordinarily positive results of these two dissertations encouraged us to make the development of a user-friendly device for the automated reprocessing of flexible endoscopes the mission.

Numerous hospitations in practices and clinics and many discussions with medical professionals have confirmed to us: the need for a simple, hygienic solution that replaces manual brush cleaning is huge.
Part of the results of a dissertation guided by Prof. Dr. Exner at the Institute for Hygiene and Public Health at the University of Bonn has recently been published in a paper in the journal Hygiene & Medicine.

2. Endoscope Pre-cleaning Device

2.1. Pre-Cleaning Process

The Endoscope Pre-cleaning Device's pre-cleaning procedure is a well-orchestrated series of steps, meticulously designed to guarantee traceability, safety, and unparalleled cleanliness of flexible endoscopes:

2.1.1 Identification and Traceability:

Upon arrival at the Central Sterilization Department (CSSD), the endoscope is registered for traceability through barcode or RFID scanning. This step ensures a meticulous record of each device for quality control and compliance.

2.1.2 Leakage Testing:

A crucial stage, an integrated leakage tester, is connected to the endoscope to confirm its structural integrity. This step safeguards against damage or leakage that could compromise the cleaning process.

2.1.3 Submersion:

After a successful leakage test and blockage check, the user is permitted to submerge the endoscope, initiating the Endoscope Pre-cleaning Device's pre-cleaning procedure. This initial immersion sets the stage for the Endoscope Pre-cleaning Device's advanced cleaning cycle.

2.1.4 Connector Set Matching:

The Endoscope Pre-cleaning Device offers intuitive guidance for selecting the correct connector set that matches the specific endoscope type. Visual graphics on the 's display assist operators in securely connecting the endoscope.

2.1.5 Blockage Testing:

After connection and before commencing the pre-cleaning procedure, the Endoscope Pre-cleaning Device conducts a blockage test on every channel of the endoscope. This critical step ensures that all channels are free from obstructions, optimizing the effectiveness of the subsequent cleaning process.

2.1.6 COMPREX Cleaning Cycle:

At the core of the Endoscope Pre-cleaning Device's capabilities lies the COMPREX (“COMprehensive PRE-cleaning eXperience”) technology. Through precise water and air

pulsing, this revolutionary process thoroughly cleans the endoscope's intricate channels, effectively removing debris and contaminants, ensuring the highest level of cleanliness.

2.1.7 Process Pressure Maintenance:

Maintaining internal, leakage test-pressure is crucial throughout the process, preventing any potential leaks from allowing water to enter the endoscope's delicate components.

2.1.8 Successful Cleaning Cycle:

Only if the entire procedure is performed as intended will the device register and present a 'successful' cleaning cycle. At this point, the endoscope can be safely removed from the water, de-pressurized, and disconnected from the connector set.

2.2. Safety

The Endoscope Pre-cleaning Device is designed with user safety as a top priority, posing no direct safety risks to operators. However, it is essential to consider the potential impact on patient safety if the pre-cleaning procedure does not function optimally. To mitigate this risk, the Endoscope Pre-cleaning Device incorporates several crucial safeguards:

2.2.1 Blockage Checking Before Cleaning:

Before initiating the cleaning cycle, the Endoscope Pre-cleaning Device conducts thorough checks to ensure there are no blockages in the endoscope's channels. This step plays a critical role in preventing any potential issues during the cleaning process.

2.2.2 Validation through Air, Water, and Time Measurements:

The Endoscope Pre-cleaning Device employs a meticulous validation process that includes precise measurements of air, water, and time. This comprehensive approach helps control the success or failure of the cleaning cycle, ensuring it meets the required standards.

2.2.3 Mandatory Washer Disinfector Step:

Furthermore, it's important to note that all endoscopes processed through the Endoscope Pre-cleaning Device undergo a final step in the washer disinfector before being used on patients. This additional layer of safety measures minimizes any residual risk, providing an extra level of assurance for patient safety.

In summary, while the Endoscope Pre-cleaning Device prioritizes user safety, its comprehensive validation processes and the mandatory washer disinfector step are in place to minimize the risk to patients, ensuring that the cleaning cycle operates effectively and reliably.

2.3 Effectiveness

The Endoscope Pre-cleaning Device will undergo rigorous validation through a series of testing procedures, including laboratory tests, simulated real-world usage scenarios, and evaluations

within hospital settings. These assessments adhere to the ISO 15883-5 standard, a recognized guideline that defines the thresholds of cleanliness by specifying the levels of soiling that must be effectively removed to ensure cleanliness is achieved.

2.4 Data Logging

The Endoscope Pre-cleaning Device is able to collect data via USB, manual input via touchscreen, RFID reader and barcode reader (connected via USB). Furthermore, it logs every cleaning cycle and accompanying data. This data can be extracted via USB or an ethernet port connected to a healthcare facility's intranet.

When connected to the intranet, users can either have the device connected to their Hospital Information System (HIS) or connect it to a Van Vliet Medical Supply proprietary application called DocuSoft. This application allows users to view the device's status and cleaning cycles, and administrate users and/or equipment in the Endoscope Pre-cleaning Device.

3. Installation

3.1. Endoscope Pre-cleaning Device

On the back of the EPD there are two inlets, a blue and red one. Blue must be connected to the water supply and red to the air supply. The inlets are push-in couplings, please make sure that the hoses are pushed in well and secure.

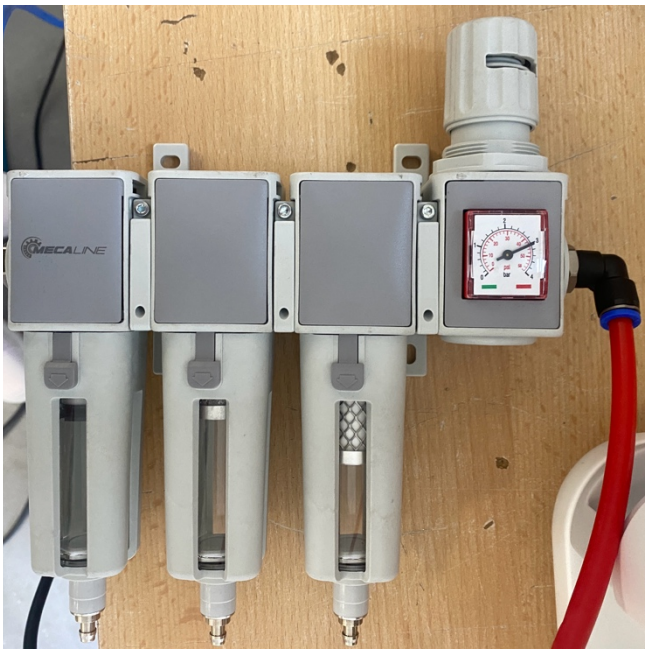
Manually, the water pressure can be adjusted. The prototype is now set on 2000 mbar (2,0 bar) on our tap water supply since this is the parameter we as Van Vliet Medical would suggest. Please check and adjust if this is different at the testing facility, this only needs to be set once during installation.

The water pressure can be adjusted by following these steps;

1. Turn on the device, wait until it is connected
2. Set the air pulse to 0 milliseconds
3. Set the water pulse to 10.000 milliseconds
4. Turn one channel on
5. Start the process
6. At the back of the prototype, pull the manual valve gently and twist higher or lower
7. Read pressure on display and keep turning till desired value
8. Press the manual valve back to secure the value
9. Reset the parameters of the air and water pulse

We have included a filter street to the package so the air supply can also be reduced before entering the EPD. This filter should be placed between the main air supply and the EPD inlet.

Same as the water pressure, the air pressure can be set to 3,0 bar by pulling the manual valve gently and twisting. When set, press the manual valve back to lock the value.



3.2. Parameters

Over the past months we have been searching for the most efficient and reliable way to test the Endoscope Pre-cleaning Device to prove the concept. For example, we ordered sheep blood from ACILA AG & ACILA Dr. Weidner GmbH to recreate the test specimen as well as the pre-fabricated test tubes used for washer disinfector validation.

During these tests, we have only been able to do a visual validation of the comprex cleaning process. After 10 pulses the hoses were visually clean and even after letting them dry over 4 days at room temperature the hoses were still easily cleaned.

In our search for the most efficient and reliable way of soil testing we came upon the Aseptium VeriTest Tags (stainless-steel soil plates). Together with the VeriTest FlexE hoses, different parameters and number of pulses were tested to find the most efficient process.

In the prototype the following parameters can be adjusted by using the display; air pressure, air pulse, water pulse and the number of pulses. On the bottom of the screen the water pressure and pulse counter are shown. The channels can be turned on or off, depending on the endoscope that is tested. The channels are separated in two blocks so different parameters can be used for the smaller or larger inner channels.

We are still assessing different sets of parameters for all diameters. For both valve sets the same parameters can be used. The two sets of parameters that we found had the best results and suggest for further testing are;

Air pressure	3000	millibar
Air pulse	2500	milliseconds
Water pulse	1500	milliseconds
Water pressure	1500	millibar
Number of pulses	50	pulses

Tab. 1. Initial parameters, Van Vliet Medical

Air pressure	3000	millibar
Air pulse	1750	milliseconds
Water pulse	1250	milliseconds
Water pressure	2000	millibar
Number of pulses	100	pulses

Tab. 2. Second set of parameters for preliminary test

Outside cleaning of the distal tip of TJF endoscopes have been a challenge in manual cleaning processes. A special connector comes with the device to improve this cleaning. The tip can be locked into the connector and the adapter fits on the "Spray Gun" output.
After cleaning the internal channels in the initial process, the distal tip process must be started.

The parameters of this process differ from the internal channel cleaning as well as the number of pulses needed to clean the tip, see table below.

NOTE: There will be a lot of water coming out of the connector. We suggest the tip is either submerged in water or the sink is covered to reduce the spraying.

Air pressure	3000	millibar
Air pulse	750	milliseconds
Water pulse	750	milliseconds
Water pressure	2000	millibar
Number of pulses	40	pulses

Tab. 3. Parameters of spray gun / distal end cleaning

4. Overview of prototype display

At the back of the product the main switch can be found. After turning the device on, it takes a couple of seconds before the display shows the main screen. Wait until the device says 'Connected' before editing the parameter. The parameters can be adjusted by selecting and pressing one, delete the initial number and insert the desired parameter.
Channels can be activated by pressing the number, when activated the channel turns blue.

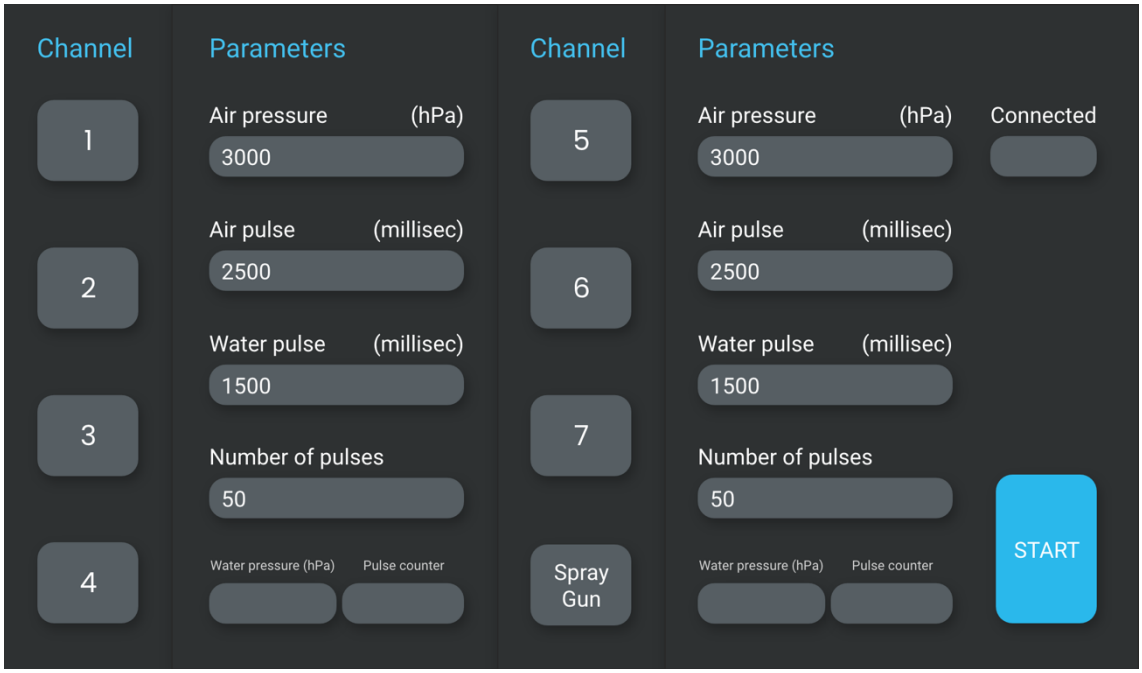


Fig. 1. Main screen

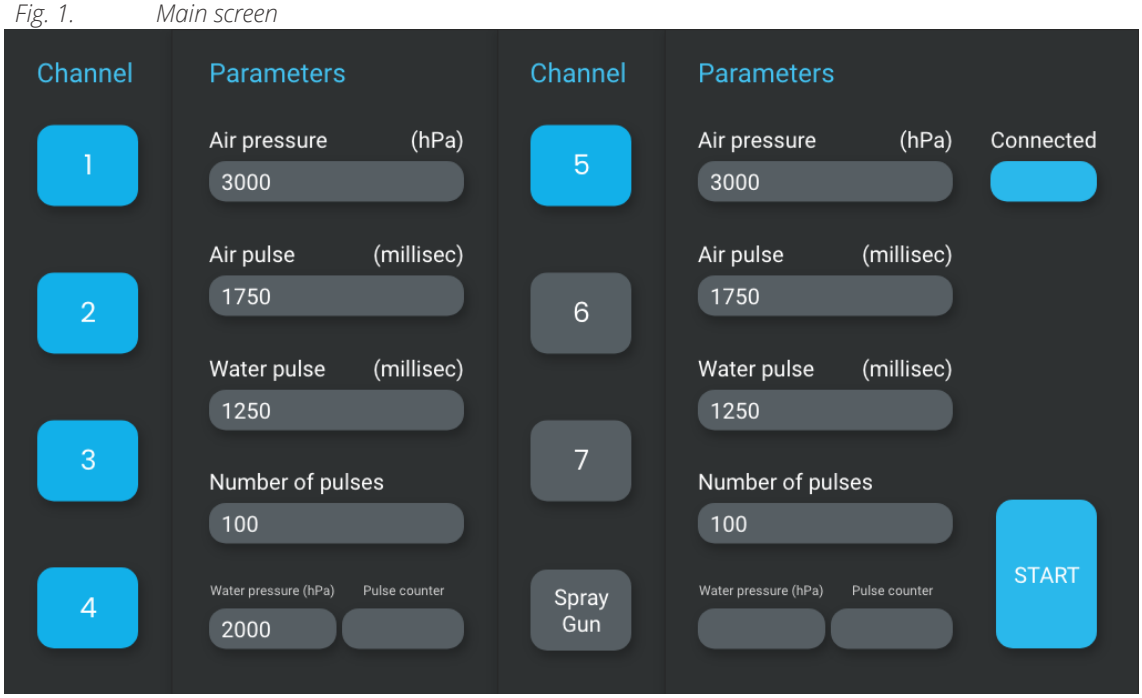


Fig. 2. Main screen, with parameters and channels activated (GF-UCT180)

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author	**Document ID**	**Revision**
Bo Koperdraat	DOC-XXXX	0

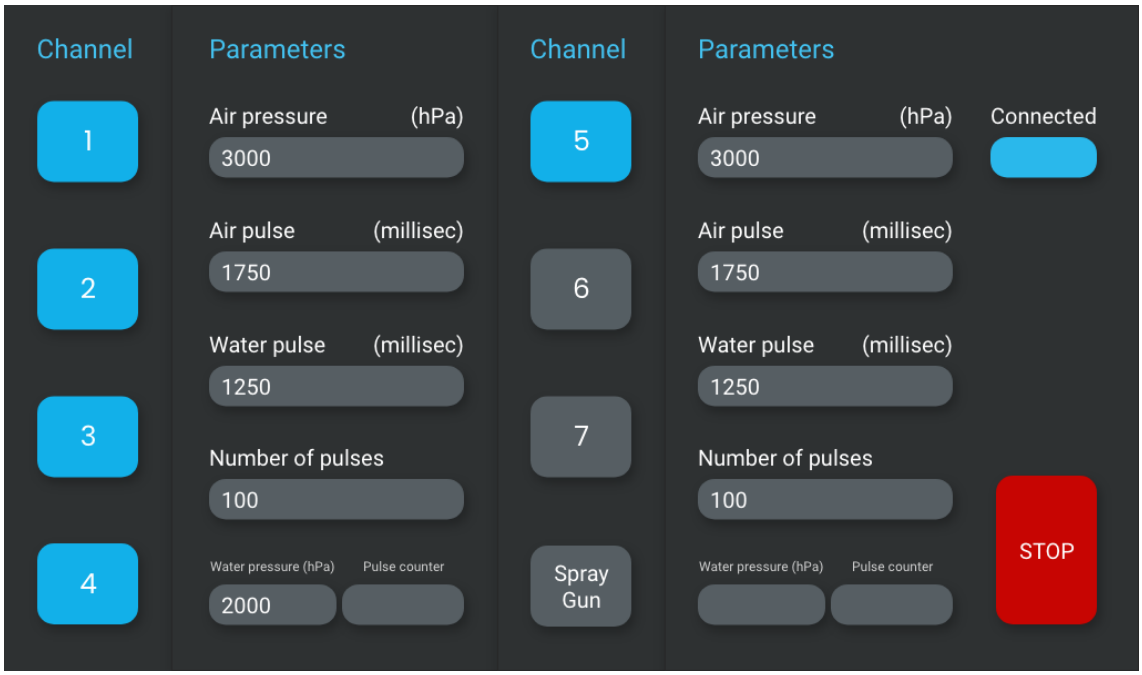


Fig. 3. Main screen, process started

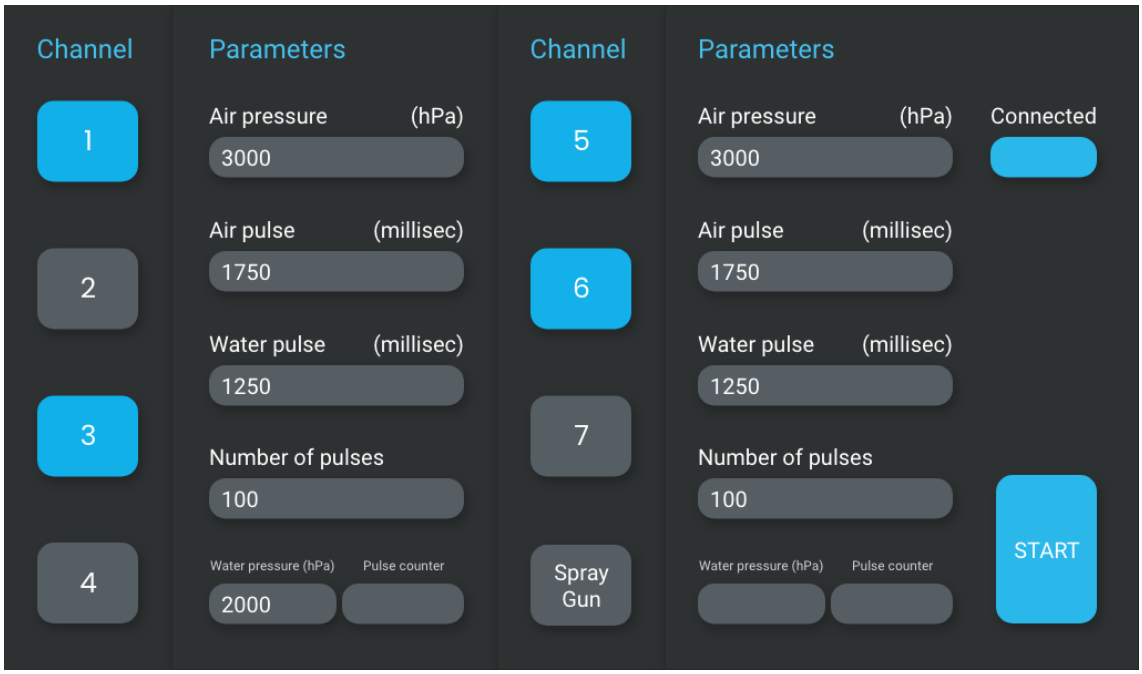


Fig. 4. Main screen, with parameters and channels activated (TJF-Q190V)

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author	**Document ID**	**Revision**
Bo Koperdraat	DOC-XXXX	0

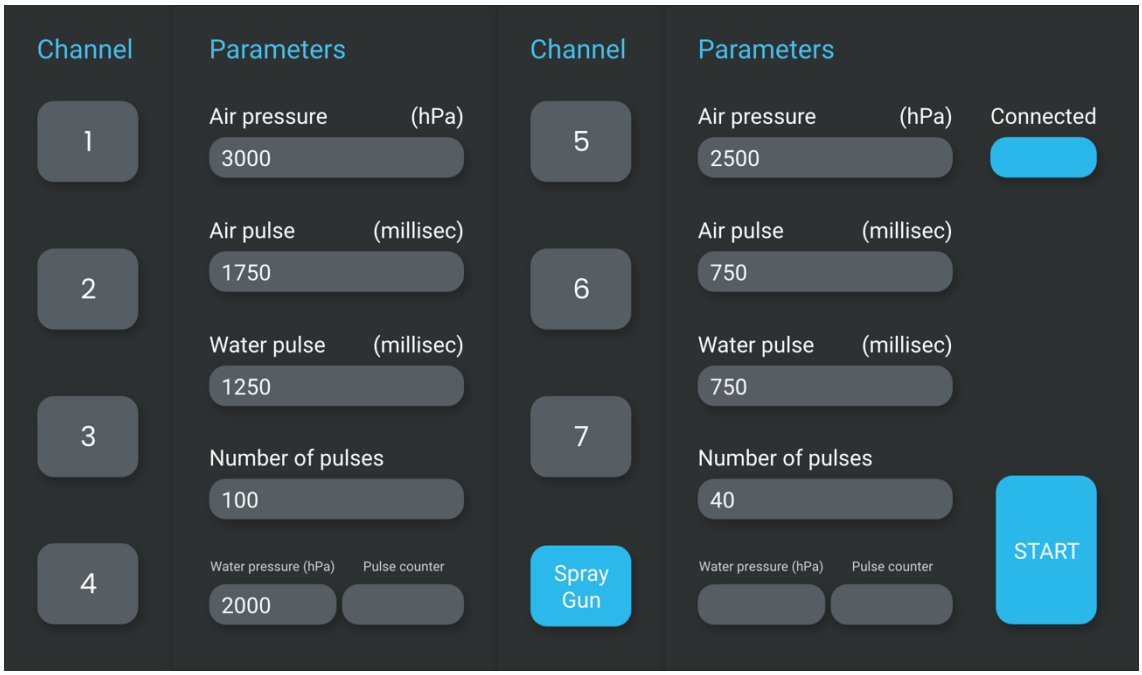


Fig. 5. Main screen, with parameters and channels activated (Spray Gun for distal tip)

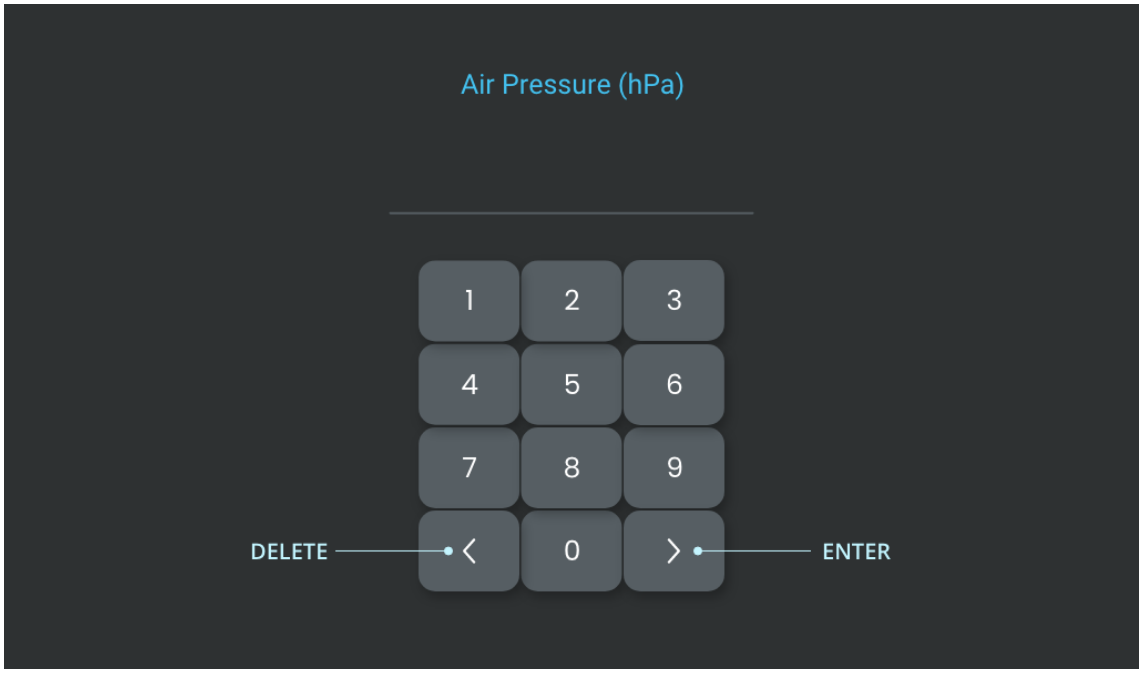


Fig. 6. Air Pressure parameter screen

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author Bo Koperdraat	**Document ID** DOC-XXXX	**Revision** 0

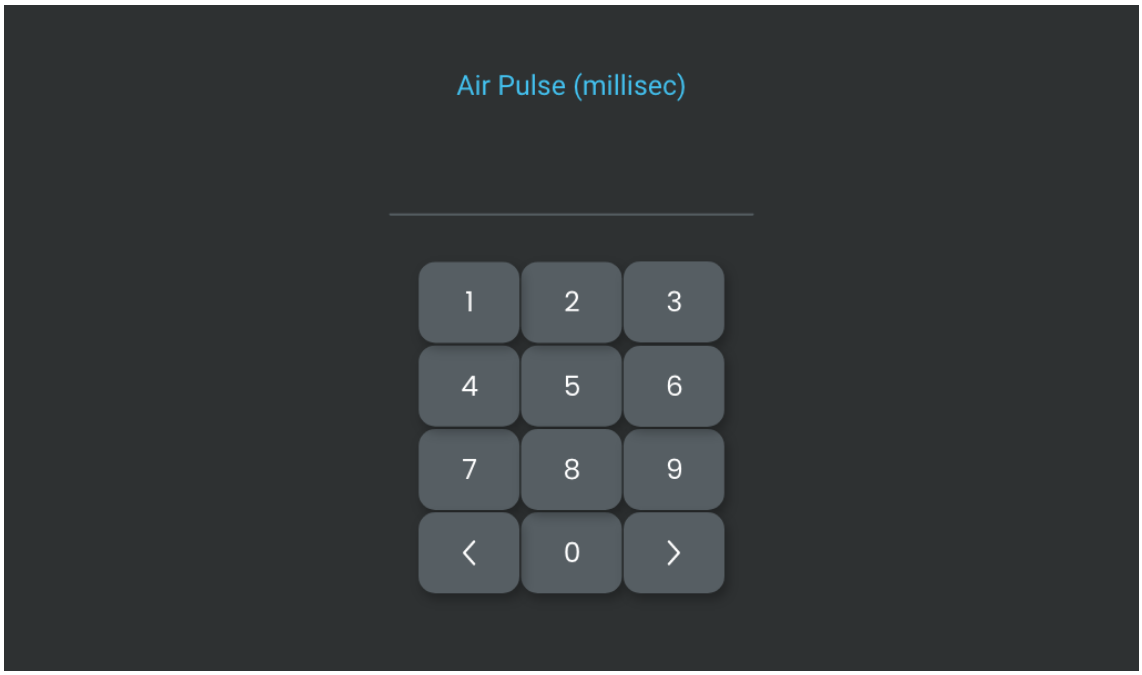


Fig. 7. Air Pulse, parameter screen

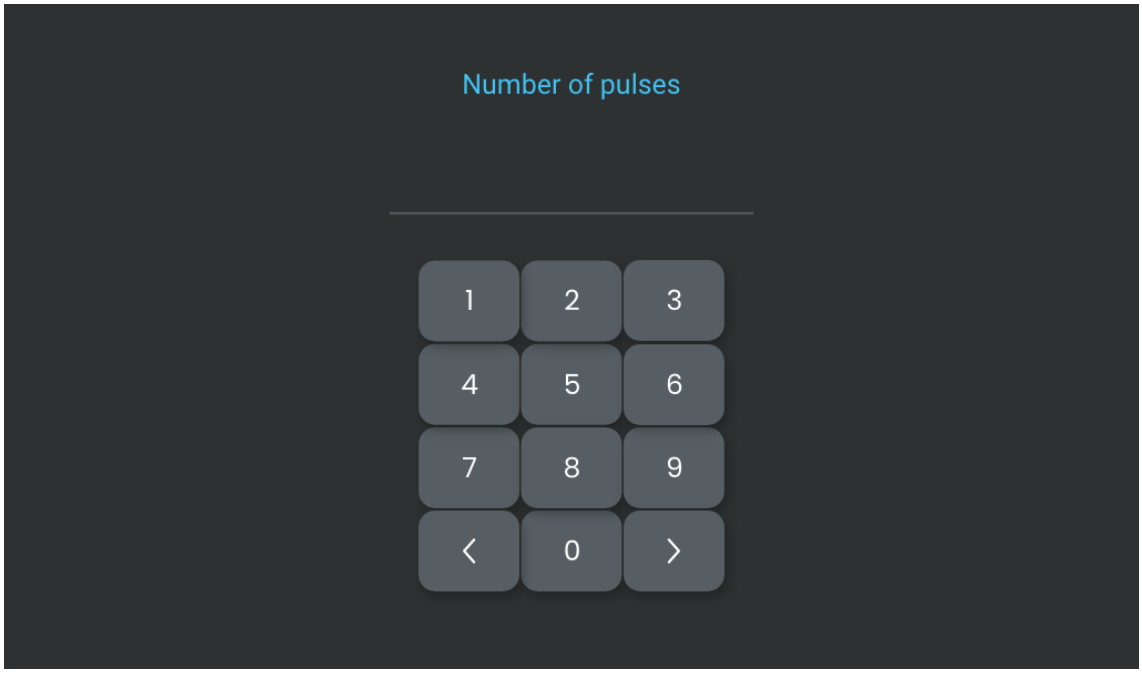


Fig. 8. Number of pulses, parameter screen

Title		Change Order #
Endoscope Pre-cleaning Device – Instructions for Use - DEMO		N/A
Author Bo Koperdraat	**Document ID** DOC-XXXX	**Revision** 0

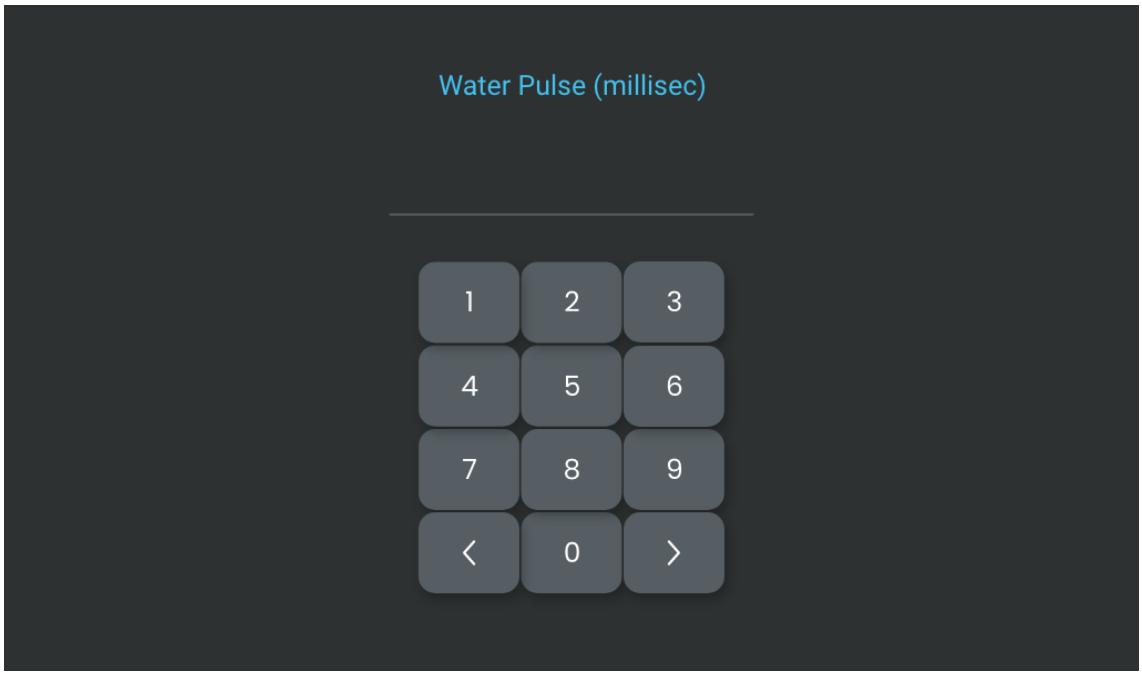


Fig. 9. Water Pulse, parameter screen

Silvia Carluccio

Firmato digitalmente da Silvia Carluccio
Data: 2025.05.08 07:31:35 +02'00'

eurofins

EVALUATION OF MECHANICAL REMOVAL OF SPORES USING ENDOSCOPE PRE-CLEANING DEVICE

Data inizio *(Started on)*. 17/02/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test *(Test strain)*	N - Inoculation suspension						Nv - Validation suspension			B filtr.		
		dilutions			cfu/ml	Log cfu/ml	dilution		cfu/ml	dilution		cfu/ml
Geobacillus stearothermophilus ATCC 7953	cfu/plate	1.00E-05	1.00E-05	1.00E-06	1.2E+07	7.06	cfu/plate	1.00E-01	3.5E+02	cfu/plate	1.00E+00	2.2E+01
	Vc1		125	16			Vc1	40		Vc1	23	
	Vc2		98	15	VALIDO (VALID)		Vc2	29	VALIDO (VALID)	Vc2	20	VALIDO (VALID)

Ceppo test (Test strain)	Positive control - Biopsy/suction channels (diameter >3mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Biopsy/suction channels (diameter >3mm)								Pc 1 SUM			
							dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177		> 1.65E+02	> 2.22	cfu/plate	1.00E-03	1.00E-04	1.00E-05		3.2E+05		9.7E+05		5.8E+07		7.76
	Case 1: Same Volume (ml) /	177				Vc (cfu/ml)	>330	32	7	residual volume	3.0	LOG(cfu/carrier)	5.99				
	Vc (cfu/plate)	>165				Vc (cfu/ml)	>330	28	4								
Ceppo test (Test strain)	Positive control - Air/Water channels (diameter of 2 mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Air/Water channels (diameter of 2 mm)								Pc 2 SUM			
							dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177		> 1.65E+02	> 2.22	cfu/plate	1.00E+00	1.00E-01	1.00E-02		1.5E+01		4.5E+01		4.0E+03		3.60
	Case 1: Same Volume (ml) /	177				Vc (cfu/ml)	4	1	1	residual volume	3.0	LOG(cfu/carrier)	1.65				
	Vc (cfu/plate)	>165				Vc (cfu/ml)	19	6	2								

Data fine sperimentazione *(Ended on)*: 19/02/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): SP 14/04/25

Sigla approvatore e data (Approver signature and date): [illegible] 14/04/25

eurofins

EVALUATION OF MECHANICAL REMOVAL OF SPORES USING ENDOSCOPE PRE-CLEANING DEVICE

Data inizio *(Started on)*. 17/02/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test (Test strain)	Negative control 1 - Biopsy/suction channels (diameter >3mm)		CFU/carrier		Negative control 1 - Air/Water channels (diameter of 2 mm)		CFU/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180		0.00E+00	Total volume (ml)	180		0.00E+00
	Case 1: Same Volume (ml) /	180			Case 1: Same Volume (ml) /	180		
	Vc (cfu/plate)	0	VALIDO (VALID)		Vc (cfu/plate)	0	VALIDO (VALID)	
Ceppo test (Test strain)	**Negative control 2 - Biopsy/suction channels (diameter >3mm)**		**CFU/carrier**		**Negative control 2 - Air/Water channels (diameter of 2 mm)**		**CFU/carrier**	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180		0.00E+00	Total volume (ml)	180		0.00E+00
	Case 1: Same Volume (ml) /	180			Case 1: Same Volume (ml) /	180		
	Vc (cfu/plate)	0	VALIDO (VALID)		Vc (cfu/plate)	0	VALIDO (VALID)	

Ceppo test (Test strain)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)		CFU/carrier	LOG(CFU/carrier)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)							Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)				Log reduction
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	> 1.65E+02	> 2.22		dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier	
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		4.0E+01		1.2E+02		7.1E+03		3.85	3.91
	Vc (cfu/plate)	>165			Vc (cfu/ml)	41	4	1	residual volume	3.0	LOG(cfu/carrier)	2.08					
					Vc (cfu/ml)	34	7	1								
Ceppo test (Test strain)	**Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)**		**CFU/carrier**	**LOG(CFU/carrier)**	**Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)**							**Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)**				**Log reduction**
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	> 1.65E+02	> 2.22		dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier	
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		0.0E+00		0.0E+00		0.0E+00		NA	3.60
	Vc (cfu/plate)	>165			Vc (cfu/ml)	0	0	0	residual volume	3.0	LOG(cfu/carrier)	NA					
					Vc (cfu/ml)	0	0	0								

Data fine sperimentazione (*Ended on*): 19/02/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): SP 14/04/25

Sigla approvatore e data (Approver signature and date): SR 14/04/25

Data inizio *(Started on)*: 11/03/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test *(Test strain)*	N - Inoculation suspension						Nv - Validation suspension			B filtr.		
		dilutions			cfu/ml	Log cfu/ml	dilution		cfu/ml	dilution		cfu/ml
Geobacillus stearothermophilus ATCC 7953	cfu/plate	1.00E-05	1.00E-05	1.00E-06	1.6E+07	7.20	cfu/plate	1.00E-01	3.8E+02	cfu/plate	1.00E+00	2.9E+01
	Vc1		163	17			Vc1	34		Vc1	31	
	Vc2		153	19	VALIDO (VALID)		Vc2	41	VALIDO (VALID)	Vc2	27	VALIDO (VALID)

Ceppo test (Test strain)	Positive control - Biopsy/suction channels (diameter >3mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Biopsy/suction channels (diameter >3mm)							Pc 1 SUM			
	Total volume (ml)	177					dilutions			cfu/ml	cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Case 1: Same Volume (ml) / Vc (cfu/plate)	177		> 1.65E+02	> 2.22	cfu/plate	1.00E-03	1.00E-04	1.00E-05		6.7E+05		2.0E+06		1.2E+08		8.08
		>165				Vc (cfu/ml)	>330	72	9	residual volume	3.0	LOG(cfu/carrier)	6.30				
						Vc (cfu/ml)	>330	66	1								

Ceppo test (Test strain)	Positive control - Air/Water channels (diameter of 2 mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Air/Water channels (diameter of 2 mm)							Pc 2 SUM			
	Total volume (ml)	177					dilutions			cfu/ml	cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Case 1: Same Volume (ml) / Vc (cfu/plate)	177		2.30E+01	1.36	cfu/plate	1.00E+00	1.00E-01	1.00E-02		1.2E+01		3.6E+01		5.9E+01		1.77
		23				Vc (cfu/ml)	13	1	1	residual volume	3.0	LOG(cfu/carrier)	1.55				
						Vc (cfu/ml)	10	0	0								

Ceppo test (Test strain)
Geobacillus stearothermophilus ATCC 7953

Data fine sperimentazione (*Ended on*): 13/03/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): [signature] 14/04/25

Sigla approvatore e data (Approver signature and date): [signature] 14/04/25

Data inizio *(Started on)*. 11/03/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test (Test strain)	Negative control 1 - Biopsy/suction channels (diameter >3mm)		CFU/carrier		Negative control 1 - Air/Water channels (diameter of 2 mm)		CFU/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180		0.00E+00	Total volume (ml)	180		0.00E+00
	Case 1: Same Volume (ml) /	180			Case 1: Same Volume (ml) /	180		
	Vc (cfu/plate)	0		VALIDO (VALID)	Vc (cfu/plate)	0		VALIDO (VALID)

Ceppo test (Test strain)	Negative control 2 - Biopsy/suction channels (diameter >3mm)		CFU/carrier		Negative control 2 - Air/Water channels (diameter of 2 mm)		CFU/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180		0.00E+00	Total volume (ml)	180		0.00E+00
	Case 1: Same Volume (ml) /	180			Case 1: Same Volume (ml) /	180		
	Vc (cfu/plate)	0		VALIDO (VALID)	Vc (cfu/plate)	0		VALIDO (VALID)

Ceppo test (Test strain)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)		CFU/carrier	LOG(CFU/carrier)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)							Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)				Log reduction
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	> 1.65E+02	> 2.22		dilutions			cfu/ml		cfu/carrier	cfu/carrier		Log cfu/carrier		3.87
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		9.1E+01		2.7E+02		1.6E+04		4.21
	Vc (cfu/plate)	>165			Vc (cfu/ml)	90	4	0	residual volume	3.0	LOG(cfu/carrier)	2.44				
					Vc (cfu/ml)	98	8	0								

Ceppo test (Test strain)	Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)		CFU/carrier	LOG(CFU/carrier)	Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)							Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)				Log reduction
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	0.00E+00	NA		dilutions			cfu/ml		cfu/carrier	cfu/carrier		Log cfu/carrier		1.29
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		1.0E+00		3.0E+00		3.0E+00		0.48
	Vc (cfu/plate)	0			Vc (cfu/ml)	1	0	0	residual volume	3.0	LOG(cfu/carrier)	0.48				
					Vc (cfu/ml)	0	0	0								

Ceppo test (Test strain)
Geobacillus stearothermophilus ATCC 7953

Data fine sperimentazione *(Ended on)*: 13/03/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): SP 14/04/25

Sigla approvatore e data (Approver signature and date): [illegible] 14/04/25

Data inizio *(Started on)*: 28/03/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test *(Test strain)*	N - Inoculation suspension						Nv - Validation suspension			B filtr.		
		dilutions			cfu/ml	Log cfu/ml	dilution		cfu/ml	dilution		cfu/ml
Geobacillus stearothermophilus ATCC 7953	cfu/plate	1.00E-05	1.00E-05	1.00E-06	1.4E+07	7.13	cfu/plate	1.00E-01	3.8E+02	cfu/plate	1.00E+00	2.9E+01
	Vc1		131	14			Vc1	34		Vc1	31	
	Vc2		141	12	VALIDO (VALID)		Vc2	41	VALIDO (VALID)	Vc2	27	VALIDO (VALID)

Ceppo test (Test strain)	Positive control - Biopsy/suction channels (diameter >3mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Biopsy/suction channels (diameter >3mm)							Pc 1 SUM			
							dilutions			cfu/ml	cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177		> 1.65E+02	> 2.22	cfu/plate	1.00E-03	1.00E-04	1.00E-05		1.0E+06		3.0E+06		1.8E+08		8.26
	Case 1: Same Volume (ml) / Vc (cfu/plate)	177				Vc (cfu/ml)	>330	104	11	residual volume	3.0	LOG(cfu/carrier)	6.48				
		>165				Vc (cfu/ml)	>330	96	10								

Ceppo test (Test strain)	Positive control - Air/Water channels (diameter of 2 mm)			CFU/carrier	LOG(CFU/carrier)	Positive control - Air/Water channels (diameter of 2 mm)							Pc 2 SUM			
							dilutions			cfu/ml	cfu/carrier		cfu/carrier		Log cfu/carrier	
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177		> 1.65E+02	> 2.22	cfu/plate	1.00E+00	1.00E-01	1.00E-02		3.3E+02		9.8E+02		8.8E+04		4.95
	Case 1: Same Volume (ml) / Vc (cfu/plate)	177				Vc (cfu/ml)	>330	37	3	residual volume	3.0	LOG(cfu/carrier)	2.99				
		>165				Vc (cfu/ml)	>330	29	3								

Ceppo test (Test strain)
Geobacillus stearothermophilus ATCC 7953

Data fine sperimentazione (*Ended on*): 31/03/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): SB 15/04/25

Sigla approvatore e data (Approver signature and date): SC 15/04/25

eurofins

EVALUATION OF MECHANICAL REMOVAL OF SPORES USING ENDOSCOPE PRE-CLEANING DEVICE

Data inizio *(Started on)*. 28/03/2025

ID. studio *(ID. Study)*: STULV24AA1951-6 GLP

ID. dispositivo (ID. device): LV-MAT-DR7G-24-297-0D83:a
ID. materiale (ID. material): LV-MAT-DR7G-24-253-0C48:a

Ceppo test (Test strain)	Negative control 1 - Biopsy/suction channels (diameter >3mm)		CFU/carrier	Negative control 1 - Air/Water channels (diameter of 2 mm)		CFU/carrier
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180	0.00E+00	Total volume (ml)	180	0.00E+00
	Case 1: Same Volume (ml) /	180		Case 1: Same Volume (ml) /	180	
	Vc (cfu/plate)	0	VALIDO (VALID)	Vc (cfu/plate)	0	VALIDO (VALID)

Ceppo test (Test strain)	Negative control 2 - Biopsy/suction channels (diameter >3mm)		CFU/carrier	Negative control 2 - Air/Water channels (diameter of 2 mm)		CFU/carrier
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	180	0.00E+00	Total volume (ml)	180	0.00E+00
	Case 1: Same Volume (ml) /	180		Case 1: Same Volume (ml) /	180	
	Vc (cfu/plate)	0	VALIDO (VALID)	Vc (cfu/plate)	0	VALIDO (VALID)

Ceppo test (Test strain)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)		CFU/carrier	LOG(CFU/carrier)	Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)								Pre-cleaning - Biopsy/suction channels (diameter >3mm) (Na)				Log reduction
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	> 1.65E+02	> 2.22		dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier		3.87
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		1.4E+02		4.1E+02		2.4E+04		4.39	
	Vc (cfu/plate)	>165			Vc (cfu/ml)	138	12	1	residual volume	3.0	LOG(cfu/carrier)	2.61					
					Vc (cfu/ml)	133	16	0									

Ceppo test (Test strain)	Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)		CFU/carrier	LOG(CFU/carrier)	Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)								Pre-cleaning - Air/Water channels (diameter of 2 mm) (Na)				Log reduction
Geobacillus stearothermophilus ATCC 7953	Total volume (ml)	177	1.10E+01	1.04		dilutions			cfu/ml		cfu/carrier		cfu/carrier		Log cfu/carrier		3.90
	Case 1: Same Volume (ml) /	177			cfu/plate	1.00E+00	1.00E-01	1.00E-02		0.0E+00		0.0E+00		1.1E+01		1.04	
	Vc (cfu/plate)	11			Vc (cfu/ml)	0	0	0	residual volume	3.0	LOG(cfu/carrier)	NA					
					Vc (cfu/ml)	0	0	0									

Data fine sperimentazione *(Ended on)*: 31/03/2025

Data verifica approvatore (approver verification date): 08/04/2025

Sigla tecnico e data (Technician signature and date): SP 15/04/25

Sigla approvatore e data (Approver signature and date): SP 15/04/25