Validation report No. 215009

Customer	Name	Enbio Group AG
	Address	Eichengasse 3 CH-4702 Oensingen, Switzerland
Sterilizer	Manufacturer	Enbio
	Type	Enbio S
	Serial No.	ST01-CH-21-10519
Tested program		134 °C FAST 134 °C 15 min 121 °C 30 min

Test facility	Address of record	Address of test laboratory
	SAL GmbH Feldstrasse 14 61479 Glashuetten	SAL GmbH Auf der Lind 10 65529 Waldems Esch

Job definition

Validation of a steam sterilization processes. The tested sterilizer, sterilization processes, products and packaging are specified by the customer.
This validation covers

- The following operational qualification tests according to EN 13060:2019: air leakage test, empty chamber test, steam penetration test with a hollow load process challenge device (EN 867-5)
- The following performance qualification tests according to EN ISO 17665-1:2006-11: physical and microbiological testing as well as dryness test of a representative load, evaluation of methods to secure the long-term stability of the process.

Study dates

02-July – 30-July-2021

Result

This validation report provides evidence that under the tested conditions, the specified processes fulfill the reviewed requirements of EN ISO 17665-1:2006-11. The limitations in chapter 1.2 have to be considered.

In the case of duplication, the test report must only be copied in its entirety.

Glashuetten, 18-Aug-2021	Glashuetten, 18 Aug 2021	Oensingen, 20-Aug-2021
Philipp Kloos SAL GmbH, Author of the report	Dr. Kerstin Kruse SAL GmbH, Auditor of the report	Arkadiusz Dorna Enbio Group AG

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Annex

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1 Introduction of the test laboratory

SAL GmbH carries out qualifications and validations in the field of reprocessing (cleaning, disinfection, sterilization). SAL GmbH is accredited by the DAkkS (German accreditation body) as a test laboratory in this field according to the directives 93/42/EEC, 90/385/EEC and the standard EN ISO/IEC 17025. The accreditation is valid for the scope specified by the annex to the accreditation certificate [D-PL-18398-02-02]. The German accreditation body is signatory to the multilateral agreements of the EA (European Co-operation for Accreditation), ILAC (International Laboratory Accreditation Cooperation) and IAF (International Accreditation Forum) for the mutual recognition of laboratory reports.

2 Task description

2.1 Scope of the validation

This report documents validation measurements of the steam sterilizer Enbio S, manufactured by Enbio Group.
The Enbio S sterilizer offers three different sterilization programs (see chapter 2.4):

- 134 °C – FAST
- 134 °C – 15 min
- 121 °C – 30 min

This validation covers

- The following operational qualification tests according to EN 13060:2019: air leakage test, empty chamber test, steam penetration test with a hollow load process challenge device (EN 867-5)
- The performance qualification of each program using a maximum load configuration consisting of reference products as specified in chapter 3.1. The performance qualification includes:
  - Dryness test for the provided products for sterilization programs 121 °C – 30 min and 134 °C – 15 min according to EN ISO 17665-1:2006
  - Microbiological testing of the sterilization effect for the three sterilization programs by the full cycle approach, as described in EN ISO 17665-1:2006 Annex D.4
- The evaluation of the methods to secure the long-term stability of the process.

The validation is based on requirements referred to by the following current standards and guidelines:

- EN ISO 17665-1:2006-11 „Sterilization of health care products - Moist heat - Part 1: Requirements for the
- development, validation and routine control of a sterilization process for medical devices".
- EN 13060:2019-02 Sterilization – Small steam sterilizers

2.2 Limitations of the validation

The validation is only valid for the sterilizer, program, load configuration and load described within this report. Changes can require a requalification and should be coordinated with the test laboratory.

The following restrictions apply:

☒ The load has to be clean.
☒ For reprocessing of medical devices, instructions of the manufacturer have to be considered.
☒ Sealed areas have to be unmounted, so that steam can reach those areas unimpeded.
☒ The reprocessing of disposables is excluded.

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2.3 Description of the sterilizer

Manufacturer	Enbio Group AG, Switzerland
Type	Enbio S
Serial number	ST01-CH-21-10519
Year of construction	2021
Chamber volume	2.7 liter
Feeding water	Demineralized water, conductivity: < 3 µS/cm
Steam supply	Internal steam generator
Conformity to standard	EN 13060

2.4 Description of the programs

134 °C FAST	preconditioning:	None
	air removal:	1 x 100 mbar
	sterilization:	3240 – 3270 mbar; 134 °C – 137 °C
		3.5 min
	drying:	1 x 600 mbar
134 °C 15 min	preconditioning:	None
	air removal:	160 → 2150 → 225 → 2150 → 225 mbar
	sterilization:	3240 – 3270 mbar; 134 °C – 137 °C
		4 min
	drying:	3 min → 160 mbar
121 °C 30 min	preconditioning:	None
	air removal:	160 → 1850 → 225 → 1850 → 225 mbar
	sterilization:	2160 – 2210 mbar; 121 °C – 124 °C
		15 min
	drying:	5 min → 120 mbar
Reference measuring position	Chamber bottom	

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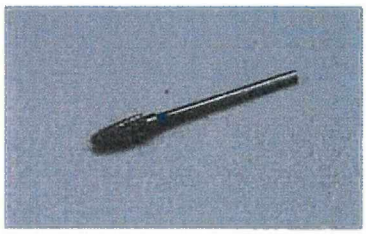
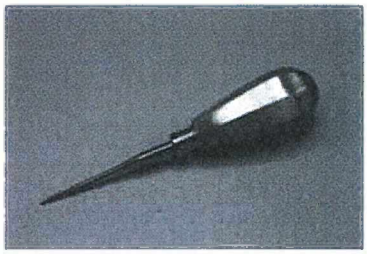
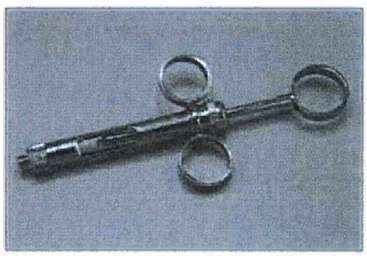
3 Material

3.1 Description of the reference load

The following representative load provided by the costumer was used for the performance qualification:

Product	Art.-No.	Description	Picture
Piercing Labret		1.2 × 8 mm, hollow	
Drill	Kristall, 31235	Solid, rough surface	
Dental Elevator	Denmax AG, AG B 108	Solid	
Dental Syringe	Denmax AG, AG J 107	Movable parts, cavities	

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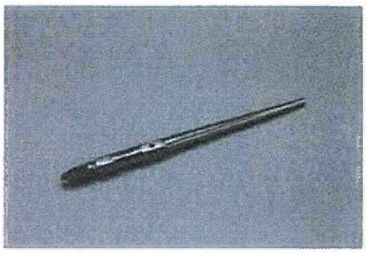
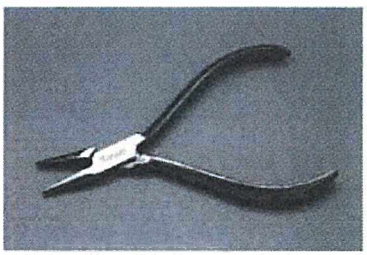
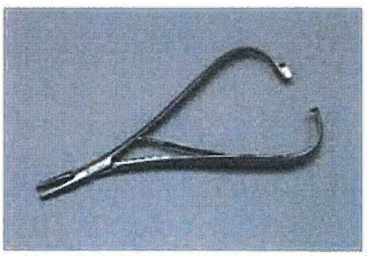
Product	Art.-No.	Description	Picture
Chisel holder/ Gouge Blade handle	LNC, LNC-281-3	Thread, cavities	
Instruments with joints: Tongs, Needle holder	Hairplay, ND 02-12, ND 03-12 and ND 06-15	Hinges	 

Packaging	Manufacturer	Sterileright Packaging
	Item number	5902340984260
		5902340984277
	Manufacturer	Medal S.C.
	Item number	STFPS6S52C90260/1W
	Manufacturer	Stericlin, VP group
	Item number	210128

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3.2 Test materials

Process challenge device

☒ Reference PCD for small steam sterilizers type B: Hollow load PCD according to EN 13060:2004 und EN 867-5:2001
Ref. 200-027, gke GmbH, Waldems

☒ Individual PCD: C-S-BMS-Dental
Ref. 200-281, gke GmbH, Waldems

Chemical indicator

☒ Integrating indicator strip according to EN ISO 11140-1 (Type 2; BMS) Charge: 1600 194300
Ref. 211-252, gke GmbH, Waldems

Measuring equipment

☒ Temperature sensors and data loggers, accuracy ± 0.1 K, TrackSense Pro, Ellab, Bockel
☒ Temperature reference, metal block calibrator, accuracy ± 0.05 K, Type Drago Basic, Isotech
☒ Preassure sensor and data logger, accuracy ± 15 mbar, TrackSense Pro, Ellab.
☒ Scale, linearity ± 0.2 g, Type 470-46, Kern, Inv.-No. 310-029 P

Spore suspension	Species	*Geobacillus stearothermophilus*
	Manufacturer	gke GmbH
	Batch	308250367
	Population	1.0×10^8 CFU/mL
	Expiry	06-2022
	$D_{121°C}$-Value	4.1 min
	Z_{Steam}-Value	9.9 °C

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4 Methods

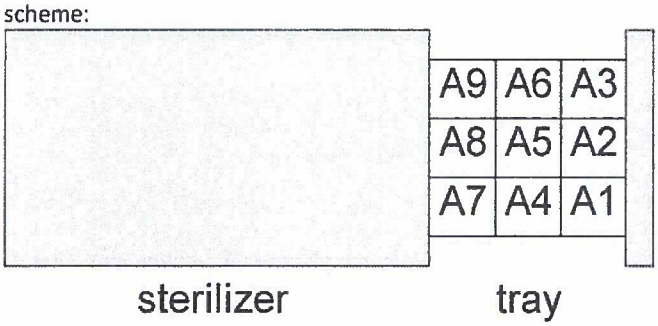
4.1 Methods for operational qualification testing

Air leakage test

The air leakage test is performed according to EN 13060, chapter 10.2.2. Pressure and temperature sensors are placed in the process chamber of the sterilizer at room temperature. The program "Vacuum leak test" is started and monitored.

Empty chamber and steam penetration test

The temperature and pressure profile in the empty process chamber is monitored with the test program BD-Test and all three sterilization programs.
The temperature sensors are placed throughout the chamber. Positions are defined according to the following scheme:



Detailed information about positioning can be found in the annex of each measurement.
Steam penetration is detected with the aid of chemical indicators, which indicate sufficient air removal and steam penetration by color change. The chemical indicators are placed in the reference process challenge device (PCD) according to EN 867-5, which represents hollow devices. Sterilizers according to EN 13060, type S are expected to pass this test.
The PCD is added to the empty chamber test. Additionally, a PCD representing a specific load, the Dental-BMS, is used.

4.2 Methods for performance qualification testing

During performance qualification, the three sterilization programs are tested with a maximum load consisting of representative products. The maximum load is to be considered the most challenging load configuration for the processes (total weight of the load: approx. 500 g).
Three consecutive runs for each program are performed and analyzed with both physical measurement and biological indicators. Temperature and pressure data loggers are used to monitor the temperature and pressure, biological indicators are used to demonstrate the effectiveness of sterilization on the products.
The evaluation of process effectiveness based on the inactivation of biological indicators is done with the full cycle approach according to EN ISO 17665-1, Annex D.

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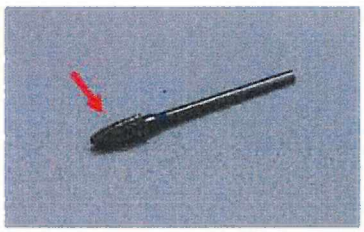
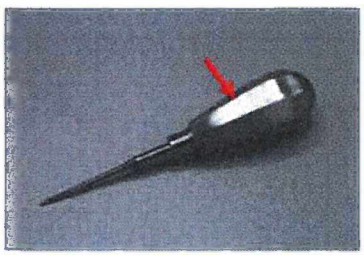
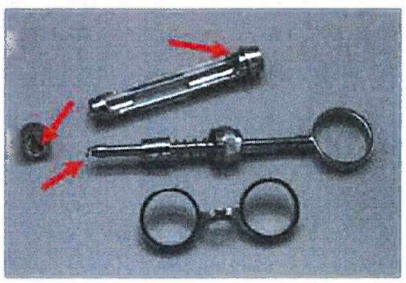
Physical measurements

Temperature sensors are placed throughout the sterilization chamber. One sensor is attached to the product with the highest mass (description of positioning: see also chapter 4.1). The pressure sensor is placed within the sterilization chamber. Temperature and pressure are recorded during each of the three tested sterilization programs.

Inoculation of products with spore suspension

Test products are inoculated with spore suspension to achieve a F_{Bio}-value of ≥ 12 min in order to perform the validation according to EN ISO 17665-1, Annex D, full cycle approach. For this, the spore suspension is diluted to a concentration of 3.3 x 10^5 CFU/mL. The products are inoculated with 10 µL spore suspension at most critical areas (see red arrows below), resulting in a F_{Bio}-value of 14.4 min for each inoculation area.
After drying, all products are single wrapped in sterile barrier systems (sterilization pouches).

Inoculation Area:

Piercing labret	In hollow area	
Carbide cutter	On rough surface	
Dental Elevator	Hand piece	
Dental Syringe[1]	Thread, Hollow lumen/ Spiral, Cylindrical shell	

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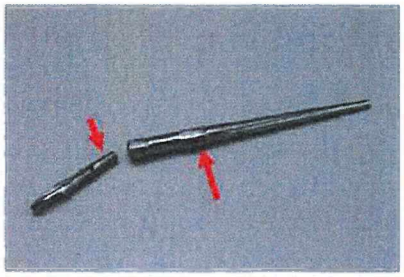
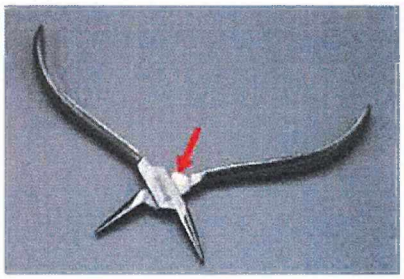
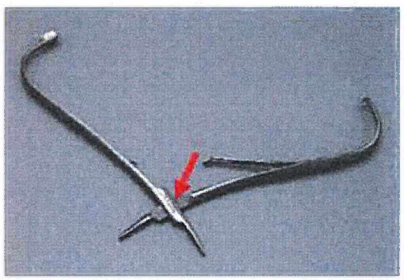
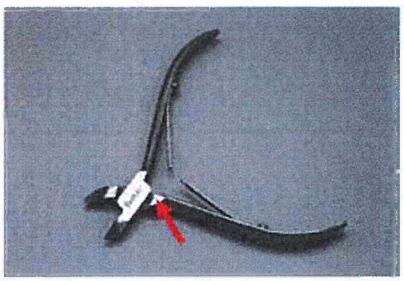
Chisel holder/ Gouge Blade handle[2]	In hollow lumen and thread	
Instruments with joints: Tongs, Needle holder	In hinges	
		
		

[1] The Dental syringe is reassembled before sterilization

[2] The Chisel holder stays disassembled during sterilization as shown in the picture

Evaluation of inoculated products' sterility

After sterilization, the products are aseptically removed from the sterilization pouches, the dental syringe is disassembled. All products are aseptically transferred into liquid growth medium (TSB) and tested for viable spores by incubation at 57 °C for 7 days.

Dryness test

The mass of the total load is measured before and after sterilization (scales, model 572, manufacturer KERN). An increase of up to +0.2 % in mass is acceptable. Additionally, product samples are checked after the sterilization process for remaining water by visual inspection.

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Dryness testing is performed only for the programs 134 °C - 15 min and 121 °C - 30 min. The program 134 °C - FAST does not include a drying phase and it is stated in the user manual that products sterilized in the program 134 °C - FAST will be wet after the process has finished.

5 Acceptance criteria and tolerances

The acceptance criteria are based on requirements of the regulations mentioned in chapter 2.1. The detailed overview is listed in the results-chapters of this report (chapters 6, 7).

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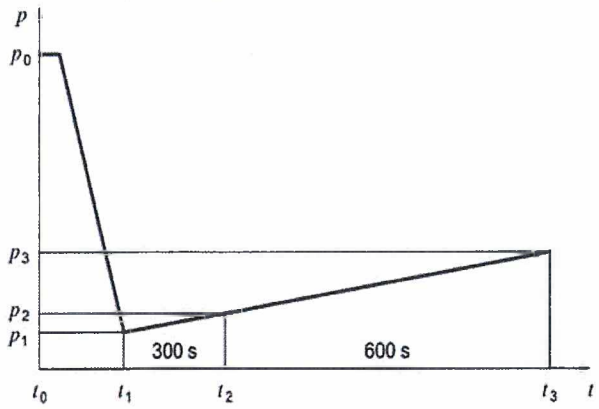
6 Results operational qualification testing

The operational qualification testing covers the vacuum leakage test, the type-test program (according to EN 13060) and the empty chamber test (according to EN ISO 17665-1).

6.1 Air leakage test

The air leakage test is performed according to EN 13060, chapter 10.2.2, using the program "Vacuum leak test".



Key

p_0 ambient atmospheric pressure
p_1 lowest pressure level, which is equal to or lower than the level set for the cycle, during the air removal and steam penetration stage
p_2 pressure after a period of 300 s
p_3 pressure after a leakage time of 600 s
t_0 start of the test
t_1 time when the pressure level is reached
t_2 start of the leakage period
t_3 end of the test

Acceptance criteria	Fulfilled? Yes	Fulfilled? No	Remarks
Requirements of EN ISO 17665-1			
The record system of the sterilizer confirms the measured process parameters	X		Annex 9
The record system of the sterilizer confirms the requirements of the process	X		Annex 2, Annex 9
Requirements of EN 13060			
Time starts at t_1 and P_1	X		Annex 2, Annex 9
$t2 - t1 = 300$ s	X		Annex 2, Annex 9
$t3 - t2 = 600$ s	X		Annex 2, Annex 9
Detection of P_2 at t_2 und P_3 at t_3			
Chamber temperature increase during $t_2 - t_3 < 3K$	X		Annex 2, Annex 9
Leakage $\frac{\Delta P}{\Delta t} = \frac{P_3 - P_2}{t_3 - t_2} < 1.3$ mbar/min	X		Annex 2, Annex 9

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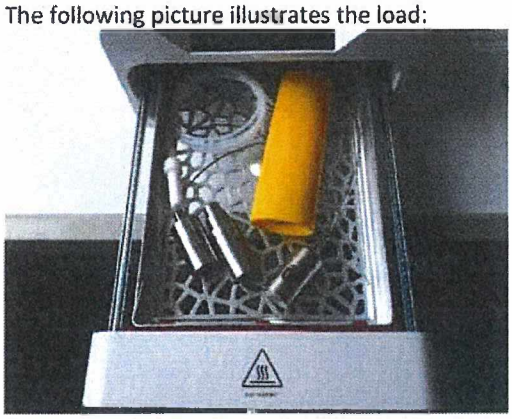
6.2 Steam penetration test and empty chamber profile according to EN 13060

The temperature and pressure profile of the empty chamber is monitored during the program BD-Test and the three sterilization programs.
The steam penetration is tested with the Dental-BMS and the reference hollow load PCD according to EN 867-5.
Steam penetration is not evaluated for the program 134 °C – FAST, as this program is only dedicated to unpackaged, solid, non-porous, simple instruments and dental tools.

The following picture illustrates the load:



Acceptance criteria	Fulfilled? Yes	Fulfilled? No	Remarks
Requirements of EN ISO 17665-1			
The record system of the sterilizer confirms the measured process parameters	X		Annex 9
The record system of the sterilizer confirms the requirements of the processes	X		Annex 2, 3, 4, 5 and 9
Steam penetration in the tested BMS	X		Annex 2, 4 and 5
Requirements of EN 13060			
Equilibration time ≤ 15 sec	X		Annex 2, 3, 4 and 5
For program "BD-Test":			
During exposure period:			
- Sterilization time (≥3:30 min)	X		Annex 2
- Minimum temperature (134 °C)	X		Annex 2
- Maximum temperature (137 °C)	X		Annex 2
- Maximum Temperature Difference (2 K)	X		Annex 2
- Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 2

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For program "134 °C - FAST":			
During exposure period:			
- Sterilization time (≥3:30 min)	X		Annex 3
- Minimum temperature (134 °C)	X		Annex 3
- Maximum temperature (137 °C)	X		Annex 3
- Maximum Temperature Difference (2 K)	X		Annex 3
- Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 3
For program "134 °C – 15 min":			
During exposure period:			
- Sterilization time (≥4:00 min)	X		Annex 4
- Minimum temperature (134 °C)	X		Annex 4
- Maximum temperature (137 °C)	X		Annex 4
- Maximum Temperature Difference (2 K)	X		Annex 4
- Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 4
For program "121 °C – 30 min":			
During exposure period:			
- Sterilization time (≥15:00 min)	X		Annex 5
- Minimum temperature (121 °C)	X		Annex 5
- Maximum temperature (124 °C)	X		Annex 5
- Maximum Temperature Difference (2 K)	X		Annex 5
- Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 5
Steam penetration of reference PCD according to EN 867-5	X		Annex 2, 4, 5*
Minimum F_0-value > 15 min	X		Annex 2, 3, 4, 5
Pressure change rate < 10 bar/min	X		Annex 2, 3, 4, 5

*: program "134 °C – FAST" has no air removal phase. This program is not to be used for hollow instruments and devices. Therefore, passing the test with the reference PCD according to EN 867-5 is not a demand for this program.

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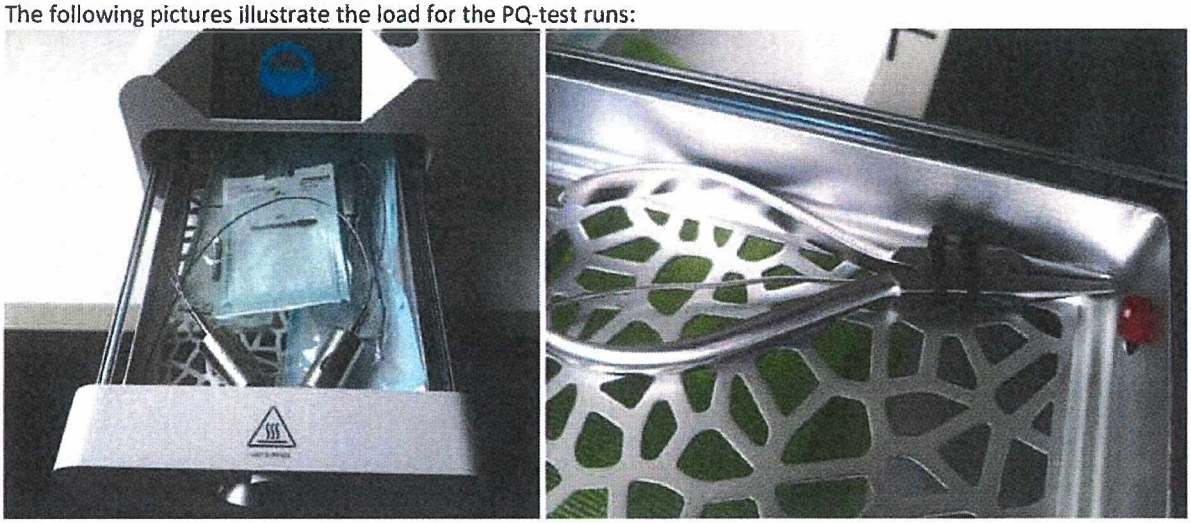
7 Results performance qualification testing

Three consecutive runs for each program are performed and analyzed with both physical measurement and biological indicators. The used maximum load consists of the inoculated products, one of each instrument listed in chapter 3.1, packaged in sterilization pouches.

The following pictures illustrate the load for the PQ-test runs:



Left: Enbio S sterilizer with maximum load. Right: Temperature measuring the surface temperature of the heaviest instrument.

7.1 Process: 134 °C – FAST

Acceptance criteria Full process	Fulfilled? Yes	No	Remarks
The record system of the sterilizer confirms the sterilization process	X		Annex 9
Pressure values according to requirements	X		Annex 3
Equilibration time ≤ 0:15 min	X		Annex 3
During exposure period:			
▪ Sterilization time (≥3:30 min)	X		Annex 3
▪ Minimum temperature (134 °C)	X		Annex 3
▪ Maximum temperature (137.0 °C)	X		Annex 3
▪ Maximum Temperature Difference (2 K)	X		Annex 3
▪ Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 3

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Dryness of the load:			
visual examination	X		Annex 8
increase in weight ≤ 0,2 %	X		Annex 8
No growth of the biological indicators	X		Annex 6

7.2 Process: 134 °C – 15 min

Acceptance criteria Full process	Fulfilled? Yes	 No	Remarks
The record system of the sterilizer confirms the sterilization process	X		Annex 9
Pressure values according to requirements	X		Annex 4
Equilibration time ≤ 0:15 min	X		Annex 4
During exposure period:			
▪ Sterilization time (≥4:00 min)	X		Annex 4
▪ Minimum temperature (134 °C)	X		Annex 4
▪ Maximum temperature (137 °C)	X		Annex 4
▪ Maximum Temperature Difference (2 K)	X		Annex 4
▪ Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 4
Dryness of the load:			
visual examination	X		Annex 8
increase in weight ≤ 0,2 %	X		Annex 8
No growth of the biological indicators	X		Annex 6

7.3 Process: 121 °C – 30 min

Acceptance criteria Full process	Fulfilled? Yes	 No	Remarks
The record system of the sterilizer confirms the sterilization process	X		Annex 9
Pressure values according to requirements	X		Annex 5

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Equilibration time ≤ 0:15 min	X		Annex 5
During exposure period:			
▪ Sterilization time (≥15:00 min)	X		Annex 5
▪ Minimum temperature (121 °C)	X		Annex 5
▪ Maximum temperature (124 °C)		X*	Annex 5, run 1 of 3, Chamber A9
▪ Maximum Temperature Difference (2 K)	X		Annex 5
▪ Comparison of the sterilization temperature with the theoretical saturated steam temperature	X		Annex 5
Dryness of the load:			
visual examination	X		Annex 8
increase in weight ≤ 0,2 %	X		Annex 8
No growth of the biological indicators	X		Annex 6

*: Over temperature was detected in test run 1 of 3 at position Chamber A9. The load did not show any over temperature. All other parameters match with requirements. This deviation is evaluated as non-critical for the safety and integrity of the sterilization process.

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8 Evaluation of the methods to secure the long-term stability of the process

Requirement	Fulfilled? Yes	Fulfilled? No	Comment
The record system of the sterilizer confirms the sterilization process	X		See Annex 9
With each batch			
• definite batch number	X		Batch number generated by the sterilizer
• used sterilization program	X		By the process control of the sterilizer
• monitoring of the parameters pressure, temperature and time	X		By check of the process protocol of the sterilizer
• sterilization control with PCD	X		C-S-BMS-Dental; REF 211-281; GKE GmbH

9 Discussion of the results

This validation covers the sterilization of stainless-steel dental and cosmetic instruments with hinges, threads, cavities and small hollow lumen, packed in sterilization pouches. The validation covers three different sterilization programs and is performed according to EN ISO 17665-1:2006-11 and EN 13060:2019-02.

The results of air leakage, steam penetration and empty chamber testing showed conformity with the requirements of EN 13060:2019-02, chapter 10.

The performance qualification with the full cycle was performed with the maximum load configuration in three consecutive runs each program to verify the reproducibility of the processes. Temperature and pressure data of these processes were analyzed. It can be confirmed that each of the three programs inactivates biological indicators with a F_{Bio}-value of 14.4 min.
It is concluded that the full sterilization cycles lead to a ≥ 12 $\log_{10}$ reduction of biological indicators with a $D_{121°C}$-value of 1 min. Therefore, the full cycle fulfills the requirements of an overkill process (EN ISO 17665-1:2006, Annex D.4) and reaches a sterilization assurance level (SAL) of ≤ 10^{-6} CFU/product.

The three tested sterilization processes are evaluated as valid for the sterilization of the tested products according to EN ISO 17665-1.

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