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Project Number: 13516

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Examination Report for
Medizin-Mechanik-Nord GmbH

**Validation of a Sterilization Process
using Steam Sterilization in Pre-vacuum Mode**

(Method MD 4.0: Sterilization validation of medical products with moist heat)

Global – Limiter with silicone handle
Self – Retaining Screwdriver

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

Sponsor Address

28-Apr-2016	4179-051201	02-May-2016
Order Date	Order Number	Delivery Date of Samples
03-Jun-2016 - 14-Jun-2016	-	
Examination Period	Remarks	

Double wrapped in sterilization pouches
Sterile Barrier System

Signatures

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Revision	Date	Reason
01	05-Jul-2016	Initial compilation of the report

1 Objective of the Examination

The objective of this project was to examine the sterilization behavior of the samples listed in Table 1 in a steam sterilization process.

Acceptance Criterion

In accordance with EN ISO 14937, Annex D, a sterilization process is regarded as successful, if the number of viable microorganisms is reduced by a factor of 10^6 in half cycle mode: Sterility assurance level (SAL) of 10^{-6}.

2 Summary

The sterilization behavior of the samples listed in Table 1 was examined according to EN ISO 14937, Annex D.

Locations which are especially difficult to be sterilized according to our experience (worst case) were directly inoculated with a spore suspension of *Geobacillus stearothermophilus* ATCC 7953.

According to EN ISO 11138-3, each location was inoculated with a minimum of 10 µl spore suspension of *Geobacillus stearothermophilus* ATCC 7953 (the nominal population per inoculated location was at least 2.0 x 10^6 spores).

Prior to the sterilization examination, the recovery of the spores from the selected inoculation locations was examined.

After inoculation, the samples were double wrapped in sterilization pouches (STERIKING® flat rolls according to EN ISO 11607-1, manufactured by Wipak) according to EN ISO 11607-1.

Three steam sterilization cycles in pre-vacuum mode were performed for each sample examined in half cycle mode with the following parameters:

- Pre-vacuum phases 3
- Sterilization temperature 132 °C
- Holding time (half cycle) 1.5 min
- Drying time 1 min *)

*) In this examination only the sterilization behavior of the samples was examined. The drying time was reduced to one minute in order to minimize possible effects on the viability of the spores.

After sterilization, the spores were recovered from the inoculated locations and examined for viability. Recovery was done according to EN ISO 11737-1.

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

3 Conclusion

The samples:

Global – Limiter with silicone handle

Self – Retaining Screwdriver

double wrapped in sterilization pouches can successfully be sterilized in a pre-vacuum steam sterilization process with the following minimum parameters:

- Pre-vacuum phases 3
- Sterilization temperature 132 °C
- Holding time (full cycle) 3.0 min

The acceptance criterion described in Chapter 1 was fulfilled.

4 Material and Methods

4.1 Samples Examined

Sample No.	Identification	REF	LOT	Inoculated location
13516-01-1	Global – Limiter with silicone handle	70105	-	1
13516-01-2		70105	-	1
13516-01-3		70113	-	1
13516-01-4		70115	-	1
13516-02-1	Self – Retaining Screwdriver T15	35310	-	2, 3
13516-02-2		35310	-	2, 3
13516-02-3		35310	-	2, 3
13516-02-4		35310	-	2, 3

Table 1: Samples examined
(The samples 13516-01-4 and 13516-02-4 were used as unsterilized references)

Figure 1: Global – Limiter with silicone handle

Figure 2: Self – Retaining Screwdriver T15

Inoculated locations chosen are numbered and indicated by red arrows.

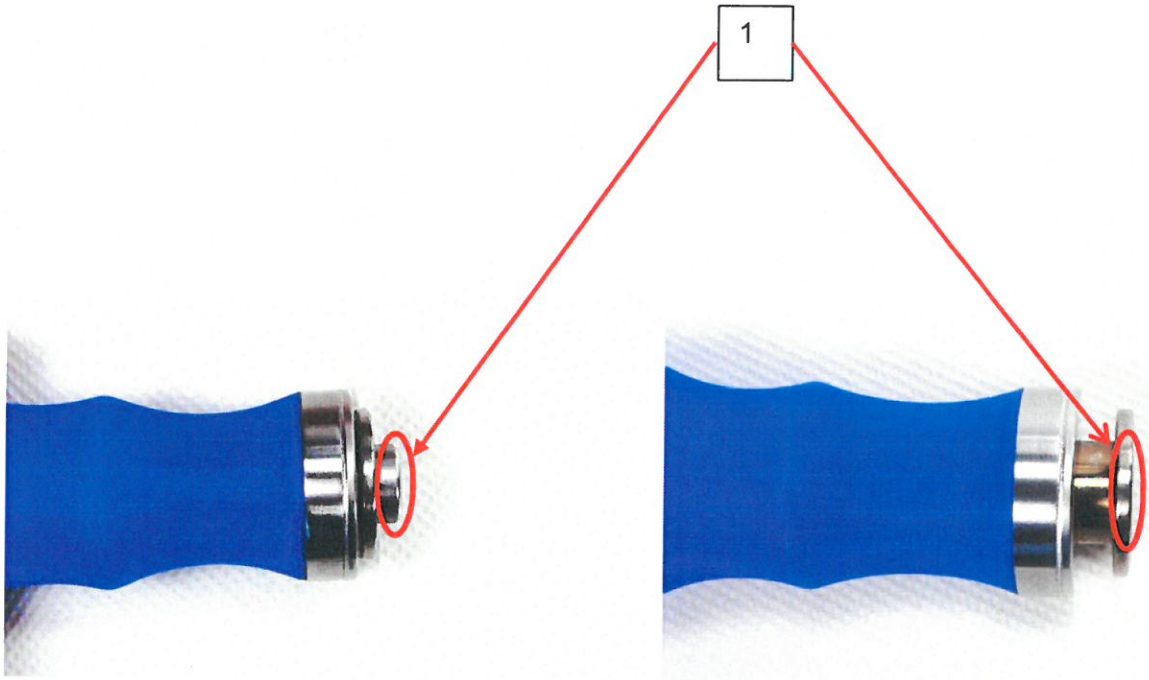


Figure 3: Global – Limiter with silicone handle
The coupling of the handle was inoculated.

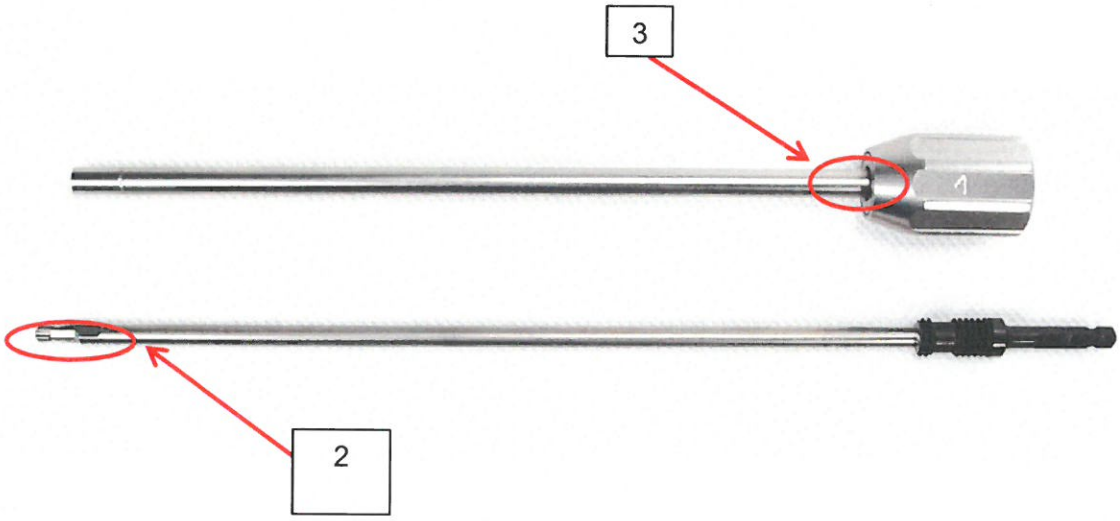


Figure 4: Self – Retaining Screwdriver T15 disassembled.
During sterilization cycle the samples were assembled.

4.2 Inoculated Locations and Recovery Methods

Sample No.	Inoculated location	Description of inoculated location	Method of recovery
13516-01-4	1	The coupling of the handle was inoculated. During the sterilization cycle the inoculated location was covered by the coupling.	1
13516-02-4	2	The tip of the screwdriver was inoculated. The sample was assembled during sterilization cycle.	5
13516-02-4	3	The gap of the sample was inoculated at the location showed in Figure 4. The sample was assembled during sterilization cycle.	1
Biological Indicator	-	-	1

Table 2: Inoculated locations and recovery methods

Method of recovery	Procedures of the standard methods of recovery
1	Two sterile cotton swabs moistened with diluent NaT were used to remove the spores from the inoculated location. The swabs were transferred into a sterile test tube with 10 ml of Tryptic Soy Broth (TSB), treated in an ultrasonic bath for 15 minutes at 40°C and then agitated for at least 10 seconds.
Method of recovery	**Procedures of the specific methods of recovery**
5	Two sterile cotton swabs moistened with diluent NaT were used to remove the spores from the inoculated location. The swabs were transferred into a sterile test tube with 10 ml of Tryptic Soy Broth (TSB), The tip of the sample was immersed also into the test tube with 10 ml of Tryptic Soy Broth (TSB) and treated in an ultrasonic bath for 15 minutes at 40°C and then agitated for at least 10 seconds. At the end the sample was removed from the test tube.

Table 3: Recovery methods applied

***Geobacillus stearothermophilus* spore suspension used for**	**LOT**	**CFU[1] / ml**	**Amount of spore suspension per inoculated location**	**Nominal population per inoculated location CFU[1]**
Recovery	2ST 10516/8-1	2.0 E+08	15 µl	3.0 E+06
Cycle[2] No. 3999				
Cycle[2] No. 4012				
Cycle[2] No. 4030				
Cycle[2] No. 4056				
Cycle[2] No. 4125				

Table 4: *Geobacillus stearothermophilus* spore suspensions used

[1] CFU: Colony forming unit
[2] Cycle: Cycle of the sterilizer

4.3 Sterile Barrier System

After inoculation and drying, the samples were double wrapped in sterilization pouches (STERIKING® flat rolls according to EN ISO 11607-1, manufactured by Wipak) and sealed with a HAWO sealing machine.

Flat rolls used for double packaging:

STERIKING® flat rolls Type R 43
STERIKING® flat rolls Type R 44

4.4 Sterilization

4.4.1 Procedure

The samples were sterilized in a steam sterilizer Selectomat HP 666-1HR (MMM). The sterilizer with an effective volume of 4 STU*) was filled-up with sieve trays containing 10 kg of hexagon-head stainless steel screws each.

*) STU: Sterilization Unit 300 mm x 300 mm x 600 mm

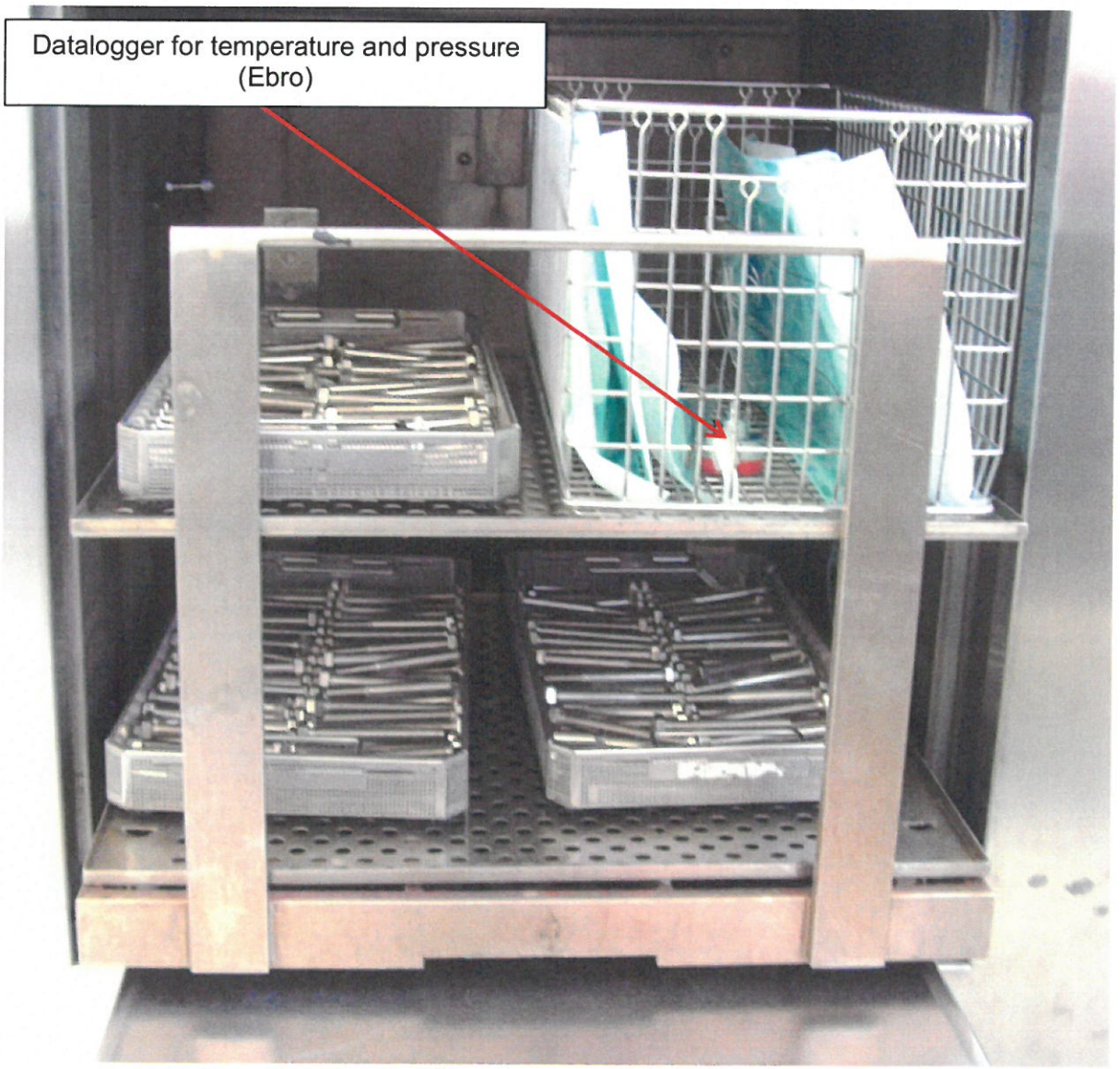


Figure 5: Load configuration within the sterilization chamber

4.4.2 Sterilization Cycles

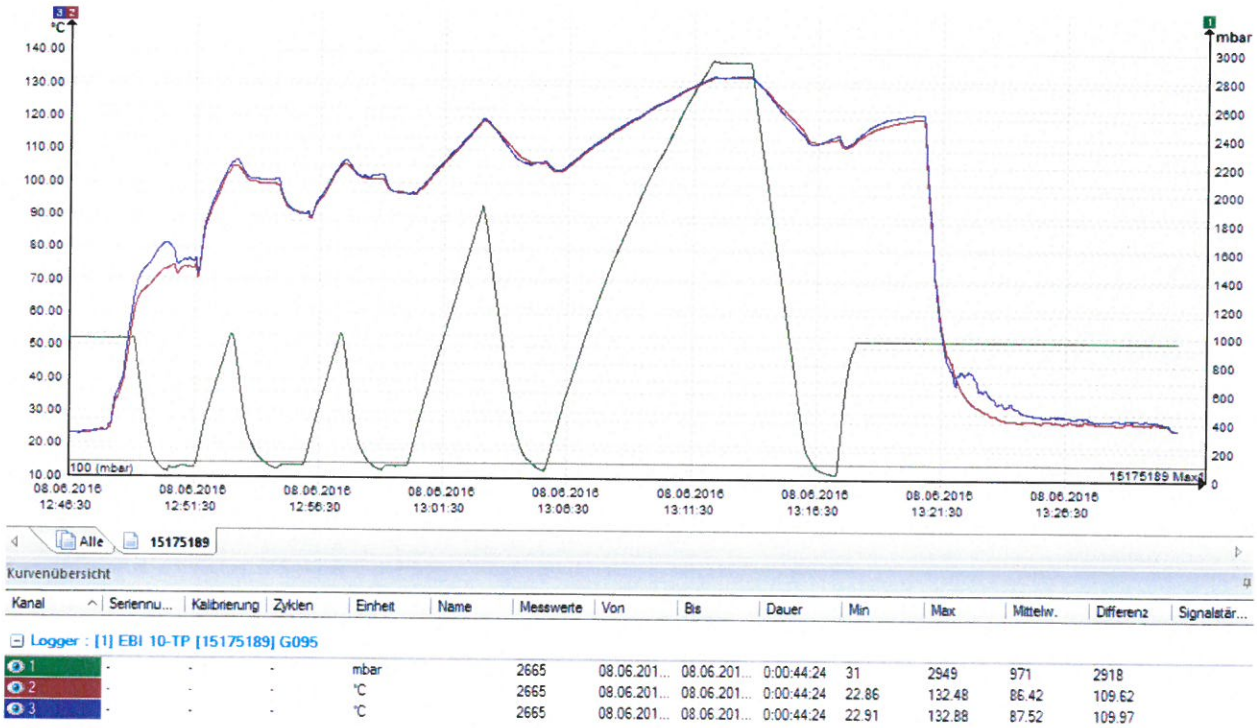
Three steam sterilization cycles in pre-vacuum mode were performed for each sample examined with the following parameters:

- Pre-vacuum phases 3
- Sterilization temperature 132 °C
- Holding time (half cycle) 1.5 min
- Drying time 1 min*)

*) In this examination only the sterilization behavior of the samples was examined. The drying time was reduced to one minute in order to minimize possible effects on the viability of the spores.

4.4.3 Monitoring the Sterilization Cycles with Logger for Pressure and Temperature

Each sterilization cycle was parametrical monitored with a logger for pressure and temperature.



Kanal	Seriennu...	Kalibrierung	Zyklen	Einheit	Name	Messwerte	Von	Bis	Dauer	Min	Max	Mittelw.	Differenz	Signalstär...
1	-	-	-	mbar		2665	08.06.201...	08.06.201...	0:00:44:24	31	2949	971	2918	
2	-	-	-	°C		2665	08.06.201...	08.06.201...	0:00:44:24	22.86	132.48	86.42	109.62	
3	-	-	-	°C		2665	08.06.201...	08.06.201...	0:00:44:24	22.91	132.88	87.52	109.97	

Figure 6: Data Logger chart of a sterilization cycle

Green line: Pressure
Red and blue lines: Temperature

4.4.4 Monitoring the Sterilization Cycles with Biological Indicators

A biological indicator was used to monitor each sterilization cycle microbiologically.

The biological indicator was prepared by inoculating a metal plate (50 mm x 15 mm x 1 mm) with a nominal amount of at least 2.0 x 10^6 CFU of *Geobacillus stearothermophilus* spores. After inoculation, the metal plate was left to dry for one hour at room temperature. After this period it was double wrapped in sterilization pouches (STERIKING® flat rolls according to EN ISO 11607-1, manufactured by Wipak), and sterilized together with the samples.

After each sterilization cycle the sterilized biological indicator was microbiologically evaluated together with the sterilized samples.

Viability of the *Geobacillus stearothermophilus* spores during sterilization was monitored with a fourth sample which was inoculated in the same manner as the samples to be examined, but not sterilized.

The unsterilized fourth sample was quantitatively microbiologically evaluated.

5 Results

5.1 Recovery

Sample No.	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-01-4	1	211	173	20	26	2.0E+06
13516-02-4	2	263	285	32	30	2.8E+06
13516-02-4	3	164	163	19	18	1.7E+06

Table 5: Microbiological results of recovery

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

5.2 Sterilization Process

5.2.1 Sterilization Cycle 1 (Cycle of the Sterilizer: 3999)

Sample No.	Inoculated location	CFU / Plate[1)]	Incubation of the TSB eluate[2)]	Identification[3)]	CFU / Inoculated location[4)]
13516-02-1	2	0	–	-	0
13516-02-2	2	0	–	-	0
13516-02-3	2	0	–	-	0
13516-02-1	3	0	–	-	0
13516-02-2	3	0	–	-	0
13516-02-3	3	0	–	-	0
Biological Indicator	-	0	–	-	0

Table 6: Microbiological results of the sterilized samples and the sterilized biological indicator

[1)] CFU of *Geobacillus stearothermophilus* on the TSA plate (1 ml of TSB eluate plated)
[2)] Result after incubation of remaining 9 ml TSB eluate for 7 days at 56±2 °C:
"+": growth of microorganisms
"–": no growth of microorganisms
[3)] Identification of *Geobacillus stearothermophilus*, if growth of microorganisms in the eluate was observed
[4)] CFU / inoculated location: CFU / TSA plate[1)] calculated for total volume of TSB eluate (10 ml)

Unsterilized Reference	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-02-4	2	94	109	15	9	1.0E+06
13516-02-4	3	>300	>300	28	36	3.1E+06

Table 7: Microbiological results of unsterilized references

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

5.2.2 Sterilization Cycle 2 (Cycle of the Sterilizer: 4012)

Sample No.	Inoculated location	CFU / Plate[1)]	Incubation of the TSB eluate[2)]	Identification[3)]	CFU / Inoculated location[4)]
13516-02-1	2	0	–	-	0
13516-02-2	2	0	–	-	0
13516-02-3	2	0	–	-	0
13516-02-1	3	0	–	-	0
13516-02-2	3	0	–	-	0
13516-02-3	3	0	–	-	0
Biological Indicator	-	0	–	-	0

Table 8: Microbiological results of the sterilized samples and the sterilized biological indicator

[1)] CFU of *Geobacillus stearothermophilus* on the TSA plate (1 ml of TSB eluate plated)
[2)] Result after incubation of remaining 9 ml TSB eluate for 7 days at 56±2 °C:
"+": growth of microorganisms
"–": no growth of microorganisms
[3)] Identification of *Geobacillus stearothermophilus*, if growth of microorganisms in the eluate was observed
[4)] CFU / inoculated location: CFU / TSA plate[1)] calculated for total volume of TSB eluate (10 ml)

Unsterilized Reference	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-02-4	2	271	244	21	24	2.5E+06
13516-02-4	3	117	127	12	18	1.2E+06

Table 9: Microbiological results of unsterilized references

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

5.2.3 Sterilization Cycle 3 (Cycle of the Sterilizer: 4030)

Sample No.	Inoculated location	CFU / Plate[1)]	Incubation of the TSB eluate[2)]	Identification[3)]	CFU / Inoculated location[4)]
13516-01-1	1	0	–	-	0
13516-01-2	1	0	–	-	0
13516-01-3	1	0	–	-	0
13516-02-1	2	0	–	-	0
13516-02-2	2	0	–	-	0
13516-02-3	2	0	–	-	0
13516-02-1	3	0	–	-	0
13516-02-2	3	0	–	-	0
13516-02-3	3	0	–	-	0
Biological Indicator	-	0	–	-	0

Table 10: Microbiological results of the sterilized samples and the sterilized biological indicator

[1)] CFU of *Geobacillus stearothermophilus* on the TSA plate (1 ml of TSB eluate plated)
[2)] Result after incubation of remaining 9 ml TSB eluate for 7 days at 56±2 °C:
"+": growth of microorganisms
"–": no growth of microorganisms
[3)] Identification of *Geobacillus stearothermophilus*, if growth of microorganisms in the eluate was observed
[4)] CFU / inoculated location: CFU / TSA plate[1)] calculated for total volume of TSB eluate (10 ml)

Unsterilized Reference	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-01-4	1	193	242	15	40	2.2E+06
13516-02-4	2	>300	>300	38	41	3.4E+06
13516-02-4	3	253	258	40	37	2.7E+06

Table 11: Microbiological results of unsterilized references

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

5.2.4 Sterilization Cycle 4 (Cycle of the Sterilizer: 4056)

Sample No.	Inoculated location	CFU / Plate[1)]	Incubation of the TSB eluate[2)]	Identification[3)]	CFU / Inoculated location[4)]
13516-01-1	1	0	–	-	0
13516-01-2	1	0	–	-	0
13516-01-3	1	0	–	-	0
Biological Indicator	-	0	–	-	0

Table 12: Microbiological results of the sterilized samples and the sterilized biological indicator

[1)] CFU of *Geobacillus stearothermophilus* on the TSA plate (1 ml of TSB eluate plated)
[2)] Result after incubation of remaining 9 ml TSB eluate for 7 days at 56±2 °C:
"+": growth of microorganisms
"–": no growth of microorganisms
[3)] Identification of *Geobacillus stearothermophilus*, if growth of microorganisms in the eluate was observed
[4)] CFU / inoculated location: CFU / TSA plate[1)] calculated for total volume of TSB eluate (10 ml)

Unsterilized Reference	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-01-4	1	96	98	8	17	1.0E+06

Table 13: Microbiological results of unsterilized reference

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

5.2.5 Sterilization Cycle 5 (Cycle of the Sterilizer: 4125)

Sample No.	Inoculated location	CFU / Plate[1)]	Incubation of the TSB eluate[2)]	Identification[3)]	CFU / Inoculated location[4)]
13516-01-1	1	0	–	-	0
13516-01-2	1	0	–	-	0
13516-01-3	1	0	–	-	0
Biological Indicator	-	0	–	-	0

Table 14: Microbiological results of the sterilized samples and the sterilized biological indicator

[1)] CFU of *Geobacillus stearothermophilus* on the TSA plate (1 ml of TSB eluate plated)
[2)] Result after incubation of remaining 9 ml TSB eluate for 7 days at 56±2 °C:
"+": growth of microorganisms
"–": no growth of microorganisms
[3)] Identification of *Geobacillus stearothermophilus*, if growth of microorganisms in the eluate was observed
[4)] CFU / inoculated location: CFU / TSA plate[1)] calculated for total volume of TSB eluate (10 ml)

Unsterilized Reference	Inoculated location	Multiplication factor 10,000		Multiplication factor 100,000		CFU[1)] recovered from inoculated location
		CFU / Plate 1	CFU / Plate 2	CFU / Plate 1	CFU / Plate 2	
13516-01-4	1	171	165	23	9	1.7E+06

Table 15: Microbiological results of unsterilized reference

[1)] CFU of *Geobacillus stearothermophilus* recovered from inoculated location, taking into account the weighted average of:
CFU on plate 1 (multiplication factor 10,000),
CFU on plate 2 (multiplication factor 10,000),
CFU on plate 1 (multiplication factor 100,000), and
CFU on plate 2 (multiplication factor 100,000)

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