This procedure is intended for laboratories performing the incubation of biological indicators (BIs). This procedure is for BI strips, spore plates and suspensions.

If a biological indicator grows after sterilization and shows positive results, it makes sense to verify the species of the growing germ to exclude mistakes of the laboratory which conducted the sterile transfer of the BI into the culture media.
For SCBIs it is not necessary to perform an analysis, as long as the sterile filter of the SCBI is intact.

The growing germs are subcultured and identified to exclude a contamination with other bacteria due to false handling of the operator.

The procedure for subculturing should be tried first on a positive sample to allow the laboratory to become familiar with the subculturing method.

Below in the subculturing procedure these steps are designed to minimize the chance of contamination.

1. Under a laminar air flow hood carefully remove the biological indicator. Place one drop of media on a slide and perform a gram stain for presumptive identification. The gram stain will appear as a Gram-positive rod. If anything other than a Gram-positive rod is observed, this would indicate contamination. No further culturing is necessary unless identification of the species of the organism is required.

2. Deposit the remaining contents of the pipette into sterile Soybean Casein Digest Broth (SCDB).

3. Incubate the media for 24 to 48 hours at a temperature of:
   37°C: indicators for EO or dry heat sterilization processes
   56°C: indicators for steam, FORM or VH_2O_2 sterilization processes

4. It is critical that the correct temperature is used to ensure the growth of *Bacillus atrophaeus* or *Geobacillus stearothermophilus.*

5. Now bacterial isolation can be performed by streaking out one inoculating loop of the SCDB growth onto prepared Soybean Casein Digest Agar (SCDA) plates and incubate at the appropriate temperature (see above no.3).

6. Additional biochemical, morphological and genetic determinations can be made to confirm the presence of *Bacillus atrophaeus* ATCC 9372 or *Geobacillus stearothermophilus* ATCC 7953 (see list below).
   It's also possible to assign a 16-S rDNA analysis (approx. 100-150 €) to identify the germ on the basis of nucleic acids.

Identification of *Bacillus Atrophaeus* ATCC* 9372 and *Geobacillus Stearothermophilus* ATCC* 7953

TEST	RESULT	
	***Bacillus Atrophaeus* ATCC* 9372**	***Geobacillus Stearothermophilus* ATCC* 7953**
1 The gram stain	+	+
Move	+	+
Catalase	+	-
Anaerobic growth	-	-
Reaction V-P	+	-
pH in the media V-P	5,4	5,2
Growth of temperature		
• minimum	10°C	40°C
• maximum	50°C	70°C
2 The reaction of egg yolk	-	
Growth with		
• lysozyme	+	-
• Aside (0,02)		-
• NaCl (5%)		-
• NaCl (7%)	+	-
• pH 5,7	+	-
Acid with		
• glucose	+	+
• arabinose	+	-
• xylose	-	-
• mannitol	+	-
Black pigment with		
• glucose	-	
• tyrosine	+	
Hydrolysis		
• starch	+	+
• hippurate – week 4	-	
Dihydroxyaceton		-
Indol		-
Using		
• citrate	+	-
• proponate	-	
Deamination phenylalanine – week 1		-
NO_2 from NO_3	+	+
Degradation		
• casein	+	+
• tyrosine	-	-

*ATCC™ is the registered Trademark of American Type Culture Collection.