Direct inoculation is only done once during validation for hollow or complex instruments when a biological indicator strip does not fit inside lumen instruments or into splits and valve parts of instruments.

10 ml *G. stearothermophilus* spore suspension 10^7 CFU/ml (art. no. 226-107) is required. The suspension shall be stored at 4-8 °C but not be frozen.

The inoculation with spore suspension can be carried out in a CSSD septically without using a microbiological lab. Use a 100 µl syringe and inject a calculated volume of 10^6 CFU (100 µl suspension of 10^7 CFU/ml) to the most critical penetration condition inside the hollow instrument and into splits and other parts which are assembled before sterilization, e.g. like valves. Then let the suspension droplet dry in the instrument for 24 hours at room temperature. Then normally wrap and sterilize. After sterilization transfer the closed pack in a microbiological lab where it is opened aseptically. The whole instrument is aseptically dismounted, all parts are put in a TSB (Tryptic Soya Broth) solution and shaken to detach the spores. The volume of TSB should be at least 9 times bigger than the instrument's volume and all parts should be submersed. If the instrument is too large or hollow, the hollow areas are flushed twice with TSB and the collected TSB is incubated.

The incubation shall be carried out with 4 TSB samples for a minimum of 7 days at 55-60 °C:

1. TSB with instruments (if sterile, no growth should occur)
2. TSB + small amount of unsterilized *G. stearothermophilus* suspension (growth shall occur) (positive control)
3. TSB without any add-on (no growth should occur) (negative control)
4. TSB with identical sterilized instrument and small amount of unsterilized *G. stearothermophilus* suspension (growth shall occur) (positive control, exclude negative effect of instrument on the growth of the BI).

Notes

1. Only if quantitative population determination is required, a validated retrieval of the spores from the instrument may be required. For normal validation procedures to check sterility, a quantitative verification is not necessary. It is sufficient that no growth is detected.

2. Tests 2-4 are only required once to be carried out in a microbiological lab if those tests are repeated.

Note: The standard „EN ISO 11737-2 Sterilization of medical devices - Microbiological methods“ provides further useful information about this subject.