The resistance of the LTSF indicators should be determined in a Resistometer with a gas atmosphere created by a formalin solution, characteristic for LTSF sterilization processes. A publication of the GKE application laboratory demonstrated that this kind of resistance determination can cause big failures (see Gömann, J., Kaiser, U., Menzel, R.: Reaction Kinetics of LTSF- Sterilization Processes. Central Steril. 2000, 8 (5): 290-293. These test conditions are not reproducible to determine the resistance of indicators. Therefore the publication proposed to test instead in the gas phase in a formalin solution and directly afterwards to neutralize the absorbed formaldehyde adhering on the indicator. This publication is available on the GKE website (www.gke.eu). This procedure has been also taken over in the standard EN ISO 11138-5.

This Standard EN ISO 11138-5 requests *Geob. stearothermophilus* spore strips with a minimum population of $10^5$ CFU/strip. The resistance has to be determined in 1-M formalin solution at 60°C. The $D_{60°C}$-value has to be at least 6 minutes. The standard EN ISO 25424 for the validation of LTSF-processes requires an $F_{Bio}$-value of 30-36 minutes at 60°C and expresses itself too unclearly, so that customers repeatedly assume that they have to comply with this $F_{Bio}$-value in this range. In fact, however, the intention of the standard must be to show 30 minutes as the minimum value for products with 10^5 CFU and 36 minutes $F_{Bio}$-value as the minimum value of 10^6 CFU products of the pharmaceutical sector. The actual D-values of biological indicators of the test germ *G. stearothermophilus* are easily 12-25 minutes and therefore by far exceed the 30-36 minutes $F_{Bio}$-value.

For sterilization monitoring the indicators are placed inside the load at locations difficult to penetrate. If hollow devices are sterilized, the use of a process challenge device (PCD) according EN 867-5 is mandatory. This standard will be replaced by EN ISO 11140-6. After sterilization the BI has to be removed from the LTSF sterilization process and incubated as follows:

1. To inactivate remaining absorbed formaldehyde, the spore strips have to be aseptically transferred in a test tube with 10 ml sterile filtrated 2 % $Na_2SO_3$-$H_2O$ solution, briefly shaken and left at room temperature for 10 minutes.

2. Afterwards the spore strips have to be aseptically transferred in a CaSO-liquid medium and heat shocked in a tightly closed test tube at 90°C for 60 minutes.

3. Using this growth medium, the spore strips have to be incubated afterwards for at least 5 days at 55-58°C as usually and have to be checked for growth every day.

For further details about resistance determination with sodium sulphite please check above mentioned publication and/or EN ISO 11138-5.

To circumvent the complex neutralization with $Na_2SO_3$ and heat shock treatment afterwards, GKE offers a self-contained biological indicator (SCBI) that can be incubated by the user without a microbiological laboratory. The SCBIs for Low Temperature Steam Formaldehyde

(LTSF) sterilization processes contain in the growth medium substances, decomposing remaining absorbed formaldehyde, so that the pretreatment with $Na_2SO_3$ according to EN ISO 11138-5 is not required anymore and the result can be obtained much faster. The SCBIs have also been designed to be used inside a GKE *Steri-Record*® Bio-Compact Process Challenge Device (Bio-C-PCD®) number 4 simulating a hollow load test according to EN 867-5 (new: EN ISO 11140-6). Furthermore the corresponding incubator is available as well.

Also in the LTSF sterilizer standard EN 14180 the PCD “Hollow load” according to EN 867-5 (new: EN ISO 11140-6) is used as a type test to check the penetration characteristics of LTSF sterilizers. It is mentioned in the standard, that the PCD should be made of plastic. Recent tests showed that stainless steel PCDs, which are more stable, don’t have any influence on the test result, since the LTSF sterilizers themselves are made of stainless steel. Therefore GKE offers all Helix- or Compact-PCD capsules for LTSF processes made of stainless steel.