	Technische Information	730-107-EN		V05
	RIT (Reduced Incubation Time) of biological indicators	Erstellt	22.11.2011	Hek
		Änderung	09.06.2021	KP
		Prüfung	09.06.2021	UK
		Freigabe	09.06.2021	UK
Ablage-Nr.: 3.0				

After a successful sterilization process the used biological indicators (BI) must not show growth after incubation. According to EN ISO 11737-2 and the pharmacopeia EP 5, 2.6.1 and USP 29 [71] for biological indicators after sterilization an incubation time of up to 14 days is required, to achieve maximum security that possibly surviving spores may show growth. For the spores *G. stearothermophilus*, *B. atrophaeus* and *B. pumilus*, which are usually used for biological indicators, incubation times of only 7 days according to EN ISO 11138-1 (7.3.2) are required.

Many biological indicators of other manufacturers however assure results after much shorter incubation times, especially for self-contained biological indicators (SCBIs). If GKE growth medium is used, this is also valid for biological indicator strips and discs.

These reduced incubation times (RIT) must have been validated, that means, it must be shown that the result of the reduced incubation time is equal to the standard incubation time of 7 or 14 days.

It has to be considered that germs used in sterilization processes may be damaged but not dead. Their repair requires a longer period and germinate much later than intact spores. This extends the incubation time and therefore a reliable evaluation. Manufacturers offering very low incubation times, e.g. 1, 3 or 8 hours, do not take this problem into consideration. Such biological indicators cannot be validated as required from a microbiological point of view. After very short incubation times (1 to 3 h) no microbiological results are evaluated but enzyme reactions, which however show the same problem, when the biological indicators are incubated later on.


FDA describes a procedure to release biological indicators where the reduced incubation time has to show the same result as a "standard" incubation over 7 days with a probability of only 97 %.

That means that biological indicators with reduced incubation time have a 97 % growth probability: Therefore after 1 to 8 hours incubation time 3 out of 100 biological indicators can grow later on. This is a Sterility Assurance Level $SAL = 3/100$ or $\sim 10^{-2}$. However, the standard EN 556-1 requires a Sterility Assurance Level of $SAL \leq 10^{-6}$.

Own data and results of other laboratories show that pre-damaged spores start growing after an incubation time of about 10 hours.

To achieve a security of 100 %, as required in the pharmacopeias, a longer incubation time is recommended. Therefore for GKE security in sterilization is more important than getting quick results and prescribes in their directions for use to incubate the steam SCBIs for 24 hours.

If a very fast evaluation is required, GKE offers a product which also contains a secondary test method, a chemical indicator (Instant-SCBI). This chemical indicator requires a longer sterilization time to show a "pass" (3 min at 134°C) than the inactivation of a biological

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indicator in the SCBI (less than 1 min at 134°C). If a "pass" of the chemical indicator is achieved, the biological indicator is killed with much higher security than the enzymatic systems of competitors.

Only in case of a very weak process used at the limit of biological indicator inactivation, the biological indicator in an Instant-SCBI must still be incubated for 24 hours, as the chemical indicator preventively indicated a fail.

The chemical indicator does not need any incubation time and can be read out immediately without any tools. → Instant-SCBI (<https://www.gke.eu/en/self-contained-bi-steam-instant-en.html>)