

<i>gke</i> - Technical Information	<b>730-107-EN</b>	
<b>RIT (<u>R</u>educed <u>I</u>ncubation <u>T</u>ime) of biological indicators</b>	<b>Version 01</b>	
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After a successful sterilization process the used biological indicators (BI) must not show growth. 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 show growth. For the spores *G. stearothermophilus*, *B. atrophaeus* and *B. pumilus* which are usually used for biological indicators, incubation times of 7 days according to EN ISO 11138-1 (7.3.2) are sufficient.

Many biological indicators, especially self-contained biological indicators (SCBIs), of other manufacturers assure results after much shorter incubation times.

However, these reduced incubation times (RIT) must have been validated, that means, it must be shown that the results of the reduced incubation time are equal to the standard incubation time of 7 or 14 days.

In sterilization processes not all spores get inactivated, but some get only damaged. The damaged ones repair themselves after some while and germinate much later than intact spores. Therefore the incubation time of damaged spores take much longer than intact ones. Manufacturers offering very low incubation time, e.g. 1, 3 or 8 hours, dismiss this problem and such BIs cannot be validated as required from a microbiological point of view. Often SCBIs are only 1-3 hours incubated using an enzyme reaction, having the same problem.

FDA prescribes a procedure to release BIs 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 3 % growth probability: Therefore after 3 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 554 requires a Sterility Assurance Level of  $SAL \leq 10^{-6}$ . Therefore such short incubation times do not conform to the required European standard.

Own data and results of other laboratories show that pre-damaged spores even first 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 *gke* describes in their directions for use to incubate the steam SCBIs for 24 hours. We believe security in sterilization is more important than getting quick results.