



1.10274.0002

Microbiology

Sterikon® plus Bioindicator

for checks on autoclaving

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Contents

Cat. No. 1.10274.0002 Pack containing 100 ampoules, each with 2 ml spore suspension

Application

Using the MERCK Sterikon® plus Bioindicator System it is possible to check the efficiency of autoclaving cycle for 15 minutes at 121 °C. Furthermore it is possible to control the sterilization success of any kind of autoclave-loading after autoclaving.

For example: pharmaceuticals, especially drugs in ampoule form, canned food, culture media; etc.

In the USP and EP the use of a bioindicator for the autoclavation control of pharmaceutical products is recommended.

Principle

The Sterikon® plus Bioindicator consists of an ampoule that contains a nutrient broth, sugar, a pH indicator and spores of a non-pathogenic organism, *Geobacillus stearothermophilus* ATCC 7953 (sporulation optimized). The thermal resistance is such that the spores are completely killed after 15 minutes, when heated in compressed steam at a temperature of 121° ± 0.5 °C (245 kPa). At lower temperatures or lower exposure times a small number of spores can survive and are capable of growing.

The ampoules are placed into the autoclave along with the batch to be autoclaved. After autoclaving, the success of the sterilization process is checked by incubation of the ampoules:

No growth of *Geobacillus stearothermophilus* indicates adequate sterilization, whereas growth shows inadequate sterilization.

Procedure

An appropriate number of ampoules are included in the batch to be autoclaved. Autoclaves with a capacity of up to 250 litres should be checked with at least 2 ampoules, whilst those with a capacity of more than 250 litres should have at least 6 ampoules.

Note: Shake each ampoule vigorously to disperse spore emidiatly before placing in the autoclave.

In order to avoid contamination by accidental breakage, it is advisable to place the ampoules in a glass beaker.

The ampoules are placed in the autoclave at sites where the most unfavourable conditions for sterilization are thought to exist, i.e. at the bottom and in the middle of the autoclave. If a single large volume of material is to be autoclaved (e.g. flasks containing a liquid), a test using the bioindicator is possible only when the ampoule is placed in the centre of the vessel in question (e.g. suspended in a flask or immersed in the contents of a tin of food). The Sterikon® plus Bioindicator can also be used to check the functional efficiency, i.e. to test whether the prescribed temperature of 121 °C is reached within the entire autoclave and whether the temperature remains constant over the whole of the prescribed period of 15 minutes.

After sterilization, the ampoules are removed and incubated 48 hours at 60° ± 2 °C. A non-sterilized ampoule should also be incubated to serve as a control. Use of the ampoules at temperatures exceeding 125 °C sterilization temperature should be avoided to prevent the possibility of damaging the bioindicator.

Evaluation

If sterilization is adequate, the *Geobacillus stearothermophilus* spores are killed off. The contents of the ampoule remains a clear red to red-violett colour.

If sterilization is inadequate, some spores of *Geobacillus stearothermophilus* survive. The contents of the ampoules then usually turn yellow to yellow-orange within 24 hours due to the formation of acid as a result of sugar fermentation and also become slightly turbid due to microbial

growth. In cases in which the spores are partially damaged, the reaction may be delayed.

The contents of the control ampoule also turn yellow to yellow-orange and become slightly turbid.

Stability

When stored at the prescribed temperature (+2° to +8 °C) in the refrigerator, the bioindicator is stable at least up to the expiry date printed on the pack.

Storage

The ampoules should be stored in the refrigerator at +2° to +8 °C. Storage at room temperature (up to approx. 25 °C) is possible for a limited period of about 1–2 weeks. Storage at temperatures exceeding +30 °C affects the product stability.

Specifications

The specifications of Sterikon® plus Bioindicator are as follows:

n = 5 x 10⁵–1 x 10⁷ spores per unit

D₁₂₁ = 1.5 to 2.0 minutes

z-Value = 7–10 °C

Acc. to the USP the heat-resistance and the number of spores are optimized, when after a sterilization time of 6 minutes at 121° ± 0.5 °C all ampoules contain living spores, whereas after 15 minutes autoclaving at 121° ± 0.5 °C all spores are dead. For the period inbetween there will be some ampoules which contain living spores and some ampoules where all spores are dead.

The spores are already in a nutrient broth.

References

I.D. Costin, J. Grigo: Bioindikatoren zur Autoklavierungskontrolle. Einige theoretische Aspekte u. praktische Erfahrungen bei der Entwicklung und Anwendung. – Zbl. Bakt. Hyg., I. Orig. A, **227**, 483–521 (1974).

H. Seyfarth: Vorschriften der USP XXIV für die Durchführung der Sterilitätsprüfung. – Pharm. Ind. **37/2**, 87–91 (1975).

J. Grigo, I.D. Costin: Vorschriften der USP XXIV für die Anwendung von Bioindikatoren zur Sterilitätskontrolle. – Pharm. Ind. **37/3**, 179–181 (1975).

N. Holstein: Untersuchungen zur Funktionsprüfung von Autoklaven mittels Bioindikatoren. – Zbl. Bakt. Hyg., I. Orig. **160**, 443–457 (1975).

United States Pharmacopoeia 23 (1995).

European Pharmacopoeia, 3rd edition 1992.

