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Microbiology

Geobacillus stearothermophilus Spore suspension

for the antibiotic sulfonamide residue test according to KUNDRAT

Contents: **Package containing 5 ampoules of 2 ml**

Application

The Geobacillus stearothermophilus spore suspension is used in conjunction with the test agar for performing the antibiotics sulfonamide residue test according to KUNDRAT, Cat. No. 1.10662. The test detects antimicrobial residues such as antibotics, sulfonamides and other chemotherapeutics in meat and other foodstuffs of animal origin. It is a routine qualitative procedure.

Principle

The test is based on agar diffusion, using spores of Geobacillus stearothermophilus as test organisms. Antibiotic and sulfonamide residues inhibit the growth of the test organism. This inhibition of growth is indicated by the formation of inhibition zones. These zones remain purple in colour whilst the rest of the nutrient medium turns yellow. Cleaning agents, disinfectants and preservatives do not influence the test.

Instruments required

Autoclave or steam bath and incubator.

Ancillary items required

Petri dishes or other nutrient vessels equipped with lids.

Filter paper discs of 6 mm diameter and capable of absorbing double their weight of water.

Reagents

Geobacillus stearothermophilus spore suspension, adjusted to a concentration of 10^8 KBE/ml (stray range: $7 \cdot 10^7$ to $3 \cdot 10^8$ KBE/ml).

Test agar for the antibiotics residue test according to KUNDRAT, Cat. No. 1.10662.

Composition	(g/l)
Peptone	17.0
Sodium chloride	3.0
D(+)glucose	3.0
Saccharose	2.0
Starch	3.0
Gelatine	2.5
Bromocresol purple	0.016
Agar-agar	10.0

Preparing the ready-to-use agar

Suspend 8.0 g of the nutrient powder in 200 ml of freshly distilled or completely demineralized water and allow to stand for 15 minutes. Boil in a water bath until completely dissolved and then autoclave for 15 minutes at 121 °C. Allow to cool to under 60 °C and add 2 ml of Geobacillus stearothermophilus spore suspension (the contents of one ampoule; shake before opening). Cast the mixture in petri dishes (5 ml per dish).

The pH of the ready-to-use solution at 25 °C: 6.8 ± 0.2.

Storage of the ready-to-use test agar

The ready-to-use test agar can be stored in air-tight petri dishes (sealed with adhesive tape) in a refrigerator (+2 to +8 °C) for up to 3 months.

Preincubated test agar (135 min. at 65 °C) can be kept under the same conditions for 1 month. It should additionally be placed in a plastic bag.

Test procedure

Wet the filter paper discs with the sample fluid or place them on sections of organ (kidney, liver) or muscle before pressing them gently onto the surface of the test agar. Up to six such discs can be used per petri dish.

Two methods can be used to carry out the test:

1. 45-minute incubation rapid test

   Preincubate the test agar for 135 min. at 65 °C. Once the discs have been added, incubate again for 45 min. at 65 °C without prediffusion.

2. 3-hour incubation

   Place the discs on the non-incubated test agar and incubate for 3 hours at 65 °C without prediffusion.

Evaluation

With the rapid test, the formation of an inhibition zone may be observed after 15–25 min. incubation period. The zones become more clearly defined on completion of the 45-minute incubation period due to the colour change that takes place. The presence of an inhibition zone should be taken as a positive result.

In the 3-hour method, only those inhibition zones with a diameter greater than 10 mm should be regarded as being positive.

Should the formation of inhibition zones be unclear after the 45-min. or 3-hour incubation period, the period can be prolonged.

Storage of Geobacillus stearothermophilus spore suspension

The suspension should be kept in a refrigerator at +2 to +8 °C. At room temperature (up to +25 °C), the suspension may be kept for 1–2 days only.

Shelf life

If kept in a refrigerator according to instructions, the test can be stored until the expiry date indicated. If used after this date, the spores may begin to lose their activity.

Literature references

Kundrat, W.: Methoden zur Bestimmung von Antibiotika-Rückständen in tierischen Produkten. – Zeitschrift f. anal. Chemie, **243**; 624 (1968).
Kundrat, W.: 45-Minuten-Schnellmethode zum mikrobiologischen Nachweis von Hemmstoffen in tierischen Produkten. – Die Fleischwirtschaft, **4**; 485–487 (1972).
Forschner, E.: Rationalisierungsmöglichkeiten beim Nachweis von Hemmstoffen in Milch im Agardiffusionsverfahren. – Archiv f. Lebensmittelhygiene, **5**; 101–104 (1972).

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Merck KGaA, 64271 Darmstadt, Germany, Tel. +49 (0)61 51 72-24 40, www.merck.de

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