Kundrat Agar

M1360

Kundrat Agar is used for the routine qualitative detection of residues from antibiotics and other chemotherapeutical agents in animal-derived food

Composition**

Ingredients	Gms / Litre
Meat peptone	7.800
Casein peptone	7.800
Yeast extract	2.800
Sodium chloride	3.000
Dextrose	1.000
Starch	4.000
Gelatin	4.000
Bromocresol purple	0.016
Agar	10.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.41 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Kundrat Agar is used for detection of antimicrobial residues in animal feed preparations. The test is carried out using a Ampoule of *Bacillus stearothermophilus* (LA840) as test microorganisms. It is also used for detection of antimicrobial residues in meat and organ samples; used together with spore suspensions of *Bacillus subtilis* (BGA) as test organism.Presence of chemotherapeutic agents is indicated by the formation of inhibition halos or zones around the disc with the sample(1).

The test is performed in the form of an agar diffusion test. Any inhibitors present produce inhibition zones devoid of bacterial growth surrounding the applied samples. With further incubation, the test organism ferments glucose present in the medium to form acid, that causes bromocresol purple to change its colour to yellow. Only the inhibition zone still retains the original violet colour of the indicators.

Test Procedure: After autoclaving the medium, cool to 50-60°C. To each 200 ml of the medium add the contents of 1 ampoule of *B.stearothermophilus* Ampoule (LA840), mix, pour in plates.

Filter paper discs with a diameter of 6 mm are soaked with the liquid specimen or placed on organ (kidney, liver) or muscle sections. The discs are then slightly pressed onto the surface of the culture medium (up to 6 discs per plate) (2).

Two methods are recommended for performing the test:

1)45 minutes incubation, rapid test:

After placing the discs on the preincubated plates, incubate them for further 45 minutes at 65°C without prediffusion.

2)3 hour incubation:

The plates are not preincubated. After the filter paper discs have been applied to the plates, they should be incubated for 3 hours at 65°C without pre-diffusion.

In the case of rapid test, formation of inhibition zones can be seen after 15-25 minutes incubation in the medium, which is otherwise turbid as a result of spore growth. After the 45 minutes incubation, the inhibition zones become even more distinct due to the fact that the culture medium changes colour. Formation of inhibition zones is to be regarded as a positive result. In

the case of the 3 hours incubation, only those inhibition zones with a diameter of more than 10 mm can be considered positive. If a distinct colour change has not occurred after 45 minutes or 3 hours, incubation can be prolonged.

Cleaning agents, disinfectants and preservatives are not covered by this test. When performing the rapid test, pre-incubating the inoculated plates enhances growth of the test organism; the inhibition zones then appear more rapidly after application of the samples.

Quality Control

Appearance
Cream to yellow coloured with green tinge, homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Light purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 4.04% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
M1360: Cultural characteristics observed after an incubation at 65°C for 18-24 hours.

Organism	Growth
Cultural Response	
Bacillus stearothermophilus ATCC 7953	good-luxuriant

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label

Reference

1.Kundrat W., 1968, Methoden zur Bestimmung von Antibiotika-Rückständen in tierischen Produkten. - Z. Anal. Chem.; 624-630.
2.Kundrat W., 1972, 45- Minuten - Schnellmethode zum mikrobiologischen Nachweis von Hemmstoffen in tierischen Produkten. - Fleischwirtsch., 52; 485-487.

Revision : 2 / 2015