Kundrat Agar, Modified, Granulated®

GM1360A

Intended Use:

Recommended for the qualitative detection of residues of antibiotics and other chemotherapeutic agents in animal derived food.

Composition**

Ingredients	g / L
Peptone	17.000
Sodium chloride	3.000
Dextrose (Glucose)	3.000
Starch	3.000
Gelatin	2.500
Bromocresol purple	0.016
Sucrose	2.000
Agar	10.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 40.52 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates or as desired.

Principle And Interpretation

Kundrat Agar, Modified is recommended for rapid or long term test defined for qualitative detection of residues of antibiotics, sulfonamides and other chemotherapeutic agents from meat and food samples. Cleaning agents, disinfectants and preservatives are not covered by this test. This method was developed by Kundrat (1,2) and is performed in the form of an agar diffusion test using a spore suspension of *Geobacillus stearothermophilus.*

This medium contains peptone, gelatin, starch and glucose which provides nitrogen, carbon compounds and other essential growth nutrients to *Geobacillus stearothermophilus*. The test organism ferments glucose and sucrose in the medium to form acid that causes bromocresol purple to change its colour from purple to yellow. Inhibitory action is seen as clearance around the inoculation zone and retains the original violet colour of the indicator.

Type of specimen

Food samples : Meat and meat products

Specimen Collection and Handling:

Test procedure:

After autoclaving the medium, cool to 50-60°C. To each 200 ml of medium add contents of 1 ampoule (LA926A) of *Geobacillus stearothermophilus* spore suspension, mix and pour a volume of 5 ml into 90 mm Petri plates (according to the German DIN 10182 Part 1). The filter paper discs with a diameter of 6 mm are soaked with liquid specimen or placed on organ (kidney, liver) or muscle sections. The discs are then gently pressed onto the surface of the culture medium . A maximum of 6 discs are placed on each plate. It is recommended to carry out preincubation of plates for 135 minutes at 65°C. Stacking of plates should be avoided so as to ensure even temperature exposure.

Either of the two methods are followed to carry out the test:

1. Rapid test, 45 minutes incubation: On placing the discs on the preincubated plates, incubate further for 45 minutes at 65°C without prediffusion.

When performing rapid test, growth of the test organism is enhanced by preincubating the inoculated plates, the inhibition zones then appear more rapidly after application of the samples.

Results of rapid test can be recorded 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 more distinct due to the change in colour of culture medium. Formation of inhibition zones is considered as a positive result.

2. Long term test, 3 hours incubation: The plates are not preincubated. On placing the filter paper discs on the plates, incubate further for 3 hours at 65°C without prediffusion.

In case of long term test, only those inhibition zones greater than 10 mm is considered as positive. If a distinct colour change

has not occurred after 45 minutes or 3 hours, it can be further incubated.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured with green tinge, homogeneous granular 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 4.05% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

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

Organism	Growth after 3-3.5h at 65°C	Colour change to yellow	Antibiotics (mcg)	Zone of inhibition
a) *Geobacillus stearothermophilus* ATCC 7953	good-luxuriant	Positive	Gentamicin (10)	18-24 mm
b) *Geobacillus stearothermophilus* ATCC 7953	good-luxuriant	positive	Gentamicin (30)	20-26 mm
c) *Geobacillus stearothermophilus* ATCC 7953	good-luxuriant	positive	Penicillin (10 IU)	35-40 mm
d) *Geobacillus stearothermophilus* ATCC 7953	good-luxuriant	positive	Streptomycin (10)	14-21 mm

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

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.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manualof Clinical Microbiology, 11th Edition. Vol. 1.

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