Kim & Rhee (KR) Agar

M2091

Intended Use

Recommended as a selective medium for the isolation of *Cronobacter* species from food samples.

Composition**

Ingredients	Gms / Litre
Peptone	10.000
Salicin	8.000
Sodium chloride	5.000
Bile salt mixture	1.500
Crystal violet	0.001
Neutral red	0.030
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 39.53 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.

Principle And Interpretation

Enterobacter species are widely distributed in nature occurring in fresh water, soil, sewage, plants, vegetables, animal and human feaces. **Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (4). This medium is recommended by APHA for the isolation of *Cronobacter* species from food samples(5). Salicin fermenting organisms produce pink to red coloured colonies whereas non-fermentors produce colourless colonies. All species of *Cronobacter* produce typical colonies and are easily distinguishable from other salicin fermenting organisms

Peptone provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Crystal violet and bile salt mixture present in the medium inhibit growth of gram-positive bacteria. Sodium chloride maintains osmotic equilibrium. Neutral red is an indicator. D-Salicin is the fermentable carbohydrate.

Key: (*)Formerly known as *Enterobacter sakazakii* .

Type of specimen

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,5,6).
After use, contaminated materials must be sterilized by autoclaving before discarding.

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. Further biochemical tests must be carried out for confirmation

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
Light yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium
Purplish red coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Cronobacter sakazakii ATCC 29544 (00214*)	50-100	luxuriant	>=50%	pink to red
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	>=50%	colourless
Staphylococcus aureus subsp. *aureus ATCC* 25923 (00034*)	>=10^4	inhibited	0%	colourless

Key: (*) Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C and prepared medium on receipt at 20-30°C. Use before expiry period 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. Use before expiry date on the label.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 (2,3).

Reference

1.American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Isenberg, (2nd Ed.), Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D.C.
3. Jorgensen,J.H., Pfaller , M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
4. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120, 1983.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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