Chromogenic Cronobacter Isolation Agar (CCI) ISO

Cat. 1446

For the isolation of presumptive Cronobacter spp. in food products and environmental samples.

Practical information

Aplications	Categories
Selective isolation	Cronobacter

Industry: Food

Regulations: ISO 22964

Principles and uses

Chromogenic Cronobacter Isolation Agar (CCI) is a selective medium for the detection of Cronobacter spp. in food products and ingredients intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling.

ISO 22964:2016 describes a horizontal method for the detection of Cronobacter spp. and recommend this medium for the isolation of Cronobacter spp.

Triptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group, essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium deoxycholate inhibits the accompanying gram positive flora. Sodium thiosulfate increase the selectivity and the recovery of Cronobacter and Enterobacter species. 5-Bromo-4-chloro-3-indolyl a-D-glucopyranoside is the chromogenic substrate.
Cronobacter (formerly Enterobacter sakazakii) is currently considered and emerging pathogen responsible for severe meningitis and necrotic enterocolitis in un-weaned babies that can be the cause of mortality rate between 40-80%.

The pathogenicity of Cronobacter for un-weaned babies' makes it necessary to review the manufacturing process of the milk-based products specialized for babies, guaranteeing the absence of the bacteria in the final product
Additional prevention measures at a hospital include the sanitary hygiene of the prepared food; reducing the time between the preparation and its administration, to impede the multiplication of microorganisms.

Formula in g/L

Bacteriological agar	15	Ferric ammonium citrate	1
Sodium chloride	5	Sodium deoxycholate	0,25
Sodium thiosulfate	1	Yeast extract	3
Tryptic digest of casein	7	5-bromo-4-chloro-3-indolyl-alpha-D-glucopyranoside	0,15

Preparation

Suspend 32,4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 ºC for 15 minutes. Cool to 50 ºC, homogenize gently and dispense into Petri dishes in amounts of 15 ml.

Instructions for use

According to ISO 22964:
- Pre-enrich the test portion in a non-selective medium such as Buffered Peptone Water BPW (Cat. 1402).
- Incubate at a temperature of 34-38 °C for 18±2 h.
- Inoculate the culture obtained in BPW in a selective medium of enrichment: Selective Broth for Cronobacter (CSB) (Cat. 2143).
- Incubate at a temperature of 41,5±1 °C for 24±2 h.
- Sow and identify the colonies in the Chromogenic Cronobacter Isolation Agar (CCI) (Cat. 1446).
- Incubate at a temperature of 41,5±1 °C for 24±2 h.
- For confirmation, typical colonies are selected from chromogenic agar, purified on a non-selective agar such as TSA (Cat. 1068) and characterized biochemically.

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Slightly amber	7,3±0,2

Microbiological test

Incubation conditions: (41,5±1 ºC / 24±2 h).
Inoculation conditions: Productivity quantitative (100±20. Min. 50 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).

Microrganisms	Specification	Characteristic reaction
Enterobacter cloacae ATCC 13047	Growth (1-2)	The colonies do not have green or greenish-blue color.
Staphylococcus aureus ATCC 25923	Total inhibition (0)	
Cronobacter sakazakii ATCC 29544	Good growth (2)	Blue-green colonies of small to medium size (1-3 mm)
Cronobacter muytjensii ATCC 51329	Good growth (2)	Blue-green colonies of small to medium size (1-3 mm)

Storage

Temp. Min.:2 ºC
Temp. Max.:8 ºC

Bibliography

ISO normative 22964:2016 Microbiology of the food chain — Horizontal method for the detection of Cronobacter spp.
GUILLAUME-Gentil, O., Sonnard, V. Kandahai, M.C., Mauragg, J.D. and Jootsen, H. A simple and Rapad Cultural Method for Detection of Enterobacter Sakazakii in environmental samples. Journal of Food. Protection, 68 (1), 2005, pp. 64-69.