Harlequin® Cronobacter Isolation Agar (NCM1008)

Intended Use

Chromogenic *Cronobacter* Isolation Agar (CCI) is recommended by ISO 22964 for the isolation and identification of *Cronobacter* spp. (formerly *Enterobacter sakazakii*), and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Although rarely causing infections in immunocompetent adults, members of the *Cronobacter* genus have been implicated in sepsis, meningitis and necrotizing enterocolitis with a high death rate in neonates. These opportunistic pathogens are common in the environment and their ability to survive desiccation presents a significant risk for post-pasteurization contamination and survival in spray dried milk products. Tryptic digest of casein and yeast extract provide a source of nitrogen and essential vitamins. The high content of sodium chloride and the presence of sodium deoxycholate produce a selective and specific medium. Microorganisms that possess α-glucosidase activity appear blue or blue-green due to the presence of the chromogen 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside. Organisms that reduce the sodium thiosulfate produce a black precipitate due to the presence of thiosulfate and ammonium iron(III) citrate. *Cronobacter* spp. possess α-D-glucosidase so appear blue to green. Similar *Enterobacteriaceae* do not express this enzyme thus grow colorless. According to ISO 22964:2017, subculture is performed after selective enrichment in *Cronobacter* Selective Broth (CSB). This medium conforms to the formulation and performance requirements of ISO 22964:2017.

Typical Formulation

Tryptic Digest of Casein	7.0 g/L
Yeast Extract	3.0 g/L
Sodium Chloride	5.0 g/L
5-Bromo-4-Chloro-3-Indolyl-α-D-Glucopyranoside	0.15 g/L
Sodium Deoxycholate	0.25 g/L
Ammonium Iron (III) Citrate	1.0 g/L
Sodium Thiosulfate	1.0 g/L
Agar	14.2 g/L

Final pH: 7.3 ± 0.2 at 25 °C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 31.6 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121 °C for 15 minutes.
4. Cool to 45-50 °C.

Test Procedure

- According to ISO 22964:2017, a test portion is pre-enriched in Buffered Peptone Water (BPW, NCM0015) at 34-38 °C for 18 h ± 2 h.
- After incubation, 0.1 mL is transferred into CSB (NCM0227) and incubated at 41.5 °C ± 1 °C for 24 h ± 2 h.
- Subculture is then performed onto CCI by means of a 10 µL loop streaked on the surface of the agar.
- CCI is incubated at 41.5 °C ± 1 °C for 24 h ± 2 h.

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- Presumptive positive colonies are then sub-cultured onto a non-selective agar (such as Tryptone Soy Agar, NCM0020) to be biochemically characterized.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing and yellow to tan.

Prepared Appearance: Prepared medium is clear to slight haze, straw to tan gel.

Minimum QC:

Cronobacter sakazakii WDCM 00214	good growth, blue-green colonies
Cronobacter muytjensii WDCM 00213	good growth, blue-green colonies
Staphylococcus aureus WDCM 00032 or WDCM 00034	total inhibition
Enterobacter cloacae WDCM 00083	weak to good growth, straw colonies

Results

Growth characteristics		
Organism	**α-D-glucosidase**	**Color**
Cronobacter spp.	+	Blue or blue-green
Enterobacter cloacae	-	Colorless or straw
Staphylococcus aureus	N/A	Inhibited

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage
Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. ISO 22964:2017 Microbiology of the food chain – Horizontal method for the detection of *Cronobacter* spp.
2. Iversen, C. (2004). A selective differential medium for *Enterobacter sakazakii*, a preliminary study. Int. J. Food Microbiol., 96, 133-139.
3. Iversen, C. (2007). Comparison of media for the isolation of *Enterobacter sakazakii*. App. Environ. Microbiol., 71(1), 48-52.