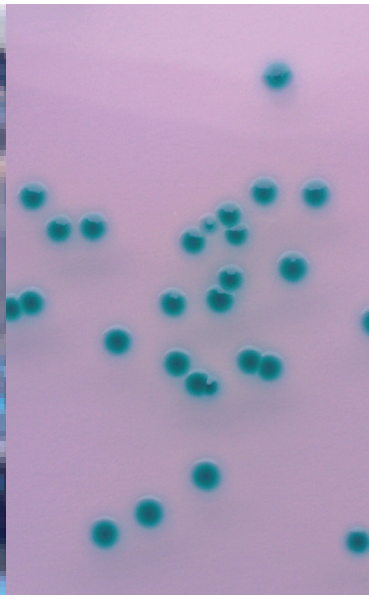
OXOID

Culture Media

SELECTIVE AND DIAGNOSTIC MEDIA FOR THE DETECTION OF CRONOBACTER SPECIES



Summary of ISO/TS 22964:2006 Milk and milk products – Detection of *Enterobacter sakazakii*

Homogenise *x* g of sample in 9 times *x* ml of Buffered Peptone Water

↓ 37°C ± 1°C for 18h ± 2h

Transfer 0.1ml of culture into 10ml of mLST/vancomycin medium

↓ 44°C ± 0.5°C for 24h ± 2h

Streak onto an Enterobacter sakazakii Isolation Agar plate

↓ 44°C ± 1°C for 24h ± 2h

Test 1-5 typical colonies for yellow colouration on TSA, oxidase, decarboxylase activity and sugar utilisation

↓

Confirmed *Cronobacter* spp.

Selective and diagnostic media for detection of *Cronobacter* species in milk powder, powdered infant formula and associated production environments.

- **Modified Lauryl Sulphate Tryptose Broth (mLST) Base CM1133**
- **Vancomycin Supplement (5mg) SR0247**
- **Enterobacter sakazakii Isolation Agar CM1134**

EASY TO READ

- Distinctive blue/green colonies on a clear background for easy identification

SELECTIVE

- Enrichment broth contains vancomycin and increased salt to inhibit the growth of non-target organisms
- Chromogenic plate differentiates *Cronobacter* spp. from other Enterobacteriaceae using an α-glucoside chromogen

FLEXIBLE

- Can also be used for environmental sampling in milk powder or infant formula factories

CONFIDENCE

- Formulations conform to ISO/TS 22964: 2006. Detection of *Enterobacter sakazakii*[1] (now known as *Cronobacter*)

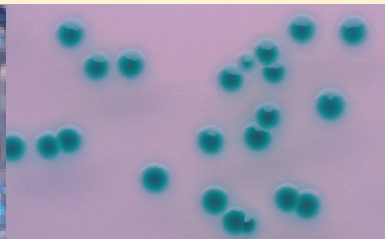
Part of our extensive range of ISO compliant media for food and environmental testing.

INTRODUCTION

Cronobacter (formerly *Enterobacter sakazakii*) are Gram-negative, rod-shaped bacteria that have been implicated in outbreaks of disease in premature infants, causing sepsis, meningitis and necrotising enterocolitis. Neurological damage can be permanent, and the death rate is reported to be as high as 40-80%[2].

Cronobacter spp. have been isolated at low levels from powdered infant formulae. The organisms' high tolerance to desiccation provides a competitive advantage in the dry environments of milk powder factories, increasing the risk of product contamination[3].

Enterobacter sakazakii was originally defined as a new species by Farmer *et al.* in 1980[4]. It was classified as a member of the genus *Enterobacter* on a phenotypic and genotypic basis and was further divided into 16 biogroups. Modern molecular techniques have allowed a more detailed analysis of the taxonomy of *Enterobacter sakazakii*, which has led to reclassification as the genus *Cronobacter*, comprising of *Cronobacter sakazakii, Cronobacter dublinensis, Cronobacter muytjensii, Cronobacter turicensis*[5], *Cronobacter malonaticus* and un-named genomospecies 1.



PRINCIPLES

The American Food and Drug Administration (FDA) published the first standardised method for isolation of *Cronobacter* spp. from dehydrated powdered infant formula[6]. It recommends incubation in sterile water followed by selective enrichment in EE broth and plating on Violet Red Bile Glucose Agar (VRBGA). Characteristic colonies on VRBGA are only presumptive Enterobacteriaceae, and so positive colonies are further streaked onto Tryptone Soya Agar, on which yellow colonies are regarded as presumptive *Cronobacter*. Therefore, including confirmatory tests, time to result can be as long as seven days.

ISO/TS 22964: 2006 Milk and milk products - Detection of *Enterobacter sakazakii* was proposed as an alternative method[1]. This comprises a non-selective enrichment in Buffered Peptone Water, selective enrichment in Modified Lauryl Sulphate Tryptose (mLST)/vancomycin medium and plating on a specific chromogenic isolation agar. This allows presumptive identification of *Cronobacter* spp. at 72 hours as opposed to 96 hours required for the FDA method.

Selective enrichment

Guillaume-Gentil *et al.*[7] proposed mLST as an improved selective enrichment broth. The salt concentration of Lauryl Sulphate Tryptose broth was increased to 34g/l, which restricted the growth of other Enterobacteriaceae and 10mg/l vancomycin was included to inhibit the growth of Gram-positive organisms commonly isolated in production environments.

Enterobacter sakazakii Isolation Agar

Unlike most members of the Enterobacteriaceae, *Cronobacter* possess the enzyme, α-glucosidase[8]. Cronobacter Isolation Agar contains a substrate for this enzyme, 5-bromo-4-chloro-3-indolyl α-D-glucopyranoside and *Cronobacter* spp. hydrolyses this chromogen to produce blue/green colonies for presumptive identification on the plate.

REFERENCES 1. ISO/TS 22964:2006 Milk and milk products - Detection of *Enterobacter sakazakii*. 2. Nazarowec-White M., Farber J.M. (1997) *Int. J. Food Microbiol.* **34**:103-113. 3. Breeuwer P., Lardeau A., Peterz M., Joosten H. M. (2003) *J. App. Microbiol.* **5**:967. 4. Farmer J.J. *et al.* (1980) *Int. J. Syst. Bacteriol.* **30**:569-584. 5. Iversen C. *et al.* (2007) *BMC Evol. Biol.* **7**:64. 6. Anonymous. (2002). U.S. Food and Drug Administration. http://www.cfsan.fda.gov/~comm/mmesakaz.html. 7. Guillaume-Gentil O. *et al.* (2005) *J. Food Prot.* **68**:64-69. 8. Muytjens H.L. (1984) *J. Clin. Microbiol.* **20**:684-686.

FORMULATION

Modified Lauryl Sulphate Tryptose/ vancomycin medium

Modified Lauryl Sulphate Tryptose Broth (mLST) Base CM1133	Grams per litre
Sodium chloride	34.0
Enzymatic digest of animal and plant tissue	20.0
Lactose	5.0
Potassium dihydrogen phosphate	2.75
Dipotassium hydrogen phosphate	2.75
Sodium lauryl sulphate	0.1
Vancomycin Supplement (5mg) SR0247E	
Vancomycin	10.0

pH 6.8 ± 0.2 at 25°C

Enterobacter sakazakii Isolation Agar CM1134	
Pancreatic peptone of casein	7.0
Yeast extract	3.0
Sodium chloride	5.0
Sodium desoxycholate	0.6
5-bromo-4-chloro-3-indolyl α-D-glucopyranoside	0.15
Crystal violet	2.0mg
Agar	12.0

pH 7.0 ± 0.2 at 25°C

ISO/TS 22964 Method	SIZE/FORMAT	ORDER CODE
Modified Lauryl Sulphate Tryptose Broth (mLST) Base	500g	CM1133B
Vancomycin Supplement (5mg)	10 vials	SR0247E
Enterobacter sakazakii Isolation Agar	500g	CM1134B
Buffered Peptone Water (ISO)	500g	CM1049B

For enrichment and isolation of *Cronobacter* spp. from milk and milk products in compliance with ISO/TS 22964:2006

In addition to the above, the Oxoid product range offers the complete solution to all your *Cronobacter* testing needs:

Alternative Products

NEW **Cronobacter Screening Broth (CSB)** a selective enrichment broth for isolation of *Cronobacter* spp. from food and environmental samples	500g	CM1121B
NEW **Chromogenic Cronobacter Isolation (CCI) Agar** an enhanced formulation of *Brilliance* E. sakazakii Agar (DFI) with improved specificity and colony colouration for the detection of *Cronobacter* spp. from food and environmental samples	500g	CM1122B

Confirmation

L-Lysine Decarboxylase Broth for differentiation of Enterobacteriaceae based on lysine activity	100 tablets (each for 5ml)	CM0308S
Simmons Citrate Agar for differentiation of Enterobacteriaceae based on citrate utilisation	500g	CM0155B
Tryptone Soya Agar for the detection of characteristic yellow colouration of *Cronobacter* spp.	500g	CM0131B
Oxidase Sticks for the detection of oxidase-positive bacteria	100 sticks	BR0064A
Microbact™ GNB 24E identification of Enterobacteriaceae and other Gram-negative bacilli; microplate format	40 tests	MB1131A
RapID™ NF Plus Panel* rapid identification of oxidase-positive Gram-negative bacilli in as little as 4 hours	20 panels	R8311005

*Check code and availability with your local Oxoid Representative

For more information about the Oxoid range of ISO compliant media and other products, please visit www.oxoid.com or talk to your local Oxoid representative.

OXOID

DEDICATED TO MICROBIOLOGY

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