



HALO13

Harlequin™ *Cronobacter sakazakii* Agar – DFI Formulation (Druggan, Forsythe & Iversen)

Description

Cronobacter sakazakii (formerly *Enterobacter sakazakii*) is a member of the *Enterobacteriaceae* family and has been associated with serious outbreak infections in neonates (premature infants) which have been fed on infant formula milk. Although rarely causing infections in immunocompetent adults, *C. sakazakii* has been implicated in sepsis, meningitis and necrotising enterocolitis with a high death rate in neonates. This opportunistic pathogen is common in the environment and its ability to survive desiccation presents a significant risk for post pasteurisation contamination and survival in spray dried milk products.

Based on the formulation described by Druggan, Forsythe and Iversen, Harlequin™ *Cronobacter sakazakii* Agar is a medium on which *Cronobacter sakazakii* appears to constitutively express high levels of α -glucosidase. This enzyme hydrolyses the chromogenic substrate 5-bromo-4-chloro-3-indolyl- α -D-glucopyranoside present in the medium, producing green coloured colonies. Other *Enterobacteriaceae* such as *E. coli* do not express strong α -glucosidase activity and appear colourless. Hydrogen sulphide producing organisms, such as *Salmonella*, and *Proteus* spp. appear grey, brown or black on this formulation due to the production of precipitated ferrous sulphate, which results from the hydrogen sulphide produced by these organisms interacting with ferric ions in the medium. This reaction prevents the weakly α -glucosidase positive *Proteus vulgaris* from appearing as green on the medium.

Selectivity is achieved from the inclusion of sodium desoxycholate which serves to inhibit the growth of most Gram-positive organisms.

Formulation

	g/litre
Tryptone	15.0
Soya peptone	5.0
Sodium chloride	5.0
Ferric ammonium citrate	1.0
Sodium desoxycholate	1.0
Sodium thiosulphate	1.0
X- α -D-glucopyranoside	0.1
Agar	15.0

Grams per litre **43.1**

Appearance

Powder: fine, free-flowing, homogeneous, buff

Finished medium: clear, straw-coloured gel

pH: 7.3 \pm 0.2

Hazard classification

NR – Not regulated

Method for reconstitution

Weigh 43.1 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix well before dispensing into Petri dishes. Dry the agar surface prior to use.

Storage

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark



Incubation

Incubate plates at $37\pm 1^{\circ}\text{C}$ for 24 hours.

Interpretation

Cronobacter sakazakii appear as green or pale green with a green 'bullseye' centre and 1-2.5mm in size.

Other organisms generally appear black (if hydrogen sulphide producers) or colourless.

References

Bowen AB, Braden CR (2006). "Invasive *Enterobacter sakazakii* disease in infants". *Emerging Infect Dis* **12** (8): 1185-9.

Caubilla-Barron J & Forsythe S (2007). "Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated infant formula". *Journal Food Protection* **13**: 467-472.

"*Enterobacter sakazakii* infections associated with the use of powdered infant formula--Tennessee, 2001" (2002). *MMWR Morb Mortal Wkly Rep* **51** (14): 297-300.

Farmer JJ III, Asbury MA, Hickman FW, Brenner DJ, the *Enterobacteriaceae* Study Group (USA) (1980). "*Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens". *Int J Syst Bacteriol* **30**: 569-84.

Iversen C, Druggan P & Forsythe S (2004). "A selective differential medium for *Enterobacter sakazakii*, a preliminary study". *International Journal of Food Microbiology* **96** (2): 133-139.

Iversen C, Lehner A, Mullane N, *et al* (2007). "The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov., *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter* genomospecies 1". *BMC Evol Biol* **7**: 64.

Iversen C, Mullane N, Barbara McCardell, *et al* (2008). "*Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov. comb. nov., *C. malonaticus* sp. nov., *C. turicensis* sp. nov., *C. muytjensii* sp. nov., *C. dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *C. dublinensis* sp. nov. subsp. *dublinensis* subsp. nov., *C. dublinensis* sp. nov. subsp. *lausannensis* subsp. nov., and *C. dublinensis* sp. nov. subsp. *lactaridi* subsp. nov.". *IJSEM*.

Lai KK (2001). "*Enterobacter sakazakii* infections among neonates, infants, children, and adults. Case reports and a review of the literature". *Medicine (Baltimore)* **80** (2): 113-22.