LAB081
CSEB (ISO)
Cronobacter sakazakii Enrichment Broth (ISO)

Modified Lauryl Sulphate Tryptose Broth Vancomycin Medium

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 lauryl sulphate tryptose broth, Cronobacter sakazakii Enrichment Broth (CSEB) has added sodium chloride for extra selectivity against competing organisms. The antibiotic vancomycin is also added to inhibit Gram-positive organisms such as *Staphylococccus aureus* which may be able to grow in this medium.

This media formulation is the secondary enrichment broth as currently recommended in the isolation protocol under ISO/TS 22964:2006(E) for the isolation of *Enterobacter sakazakii* from milk and milk products.

Formulation

	g/litre
Enzymatic digest of animal and plant tissue	20.0
Lactose	5.0
Sodium chloride	34.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen orthophosphate	2.75
Sodium lauryl sulphate	0.1
Grams per litre	**64.6**

Appearance

Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, straw liquid

pH: 6.8 ± 0.2

Hazard classification

NR – Not regulated

Method for reconstitution

Weigh 64.6 grams of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and if required, heat gently to dissolve. Dispense in 10ml volumes and sterilise by autoclaving for 15 minutes at 121°C. Cool to 47°C.

Prepare a solution of vancomycin in distilled water at a concentration of 1mg/ml. Add 0.1ml of the vancomycin solution to the sterile broth to obtain a final concentration of 0.1mg per 10ml (10mg/L) of CSEB.

Inoculation
Following pre-enrichment in Buffered Peptone Water, transfer 0.1mL of the obtained culture into 10ml LAB081 CSEB.

Incubation
Incubate at 44°C ± 0.5°C for 24 hours ± 2 hours.

Sub-culture & Interpretation
After incubation, tubes showing turbidity should be streaked onto HAL012 CSIM (ISO).

Storage

Dehydrated culture media:	10-25°C away from direct sunlight.
Prepared media (with vancomycin):	1 day at 2-8°C in the dark.

Minimum Q.C. organisms
Cronobacter sakazakii ATCC 12868
Cronobacter muytjensii ATCC 51329
Escherichia coli ATCC 25922 (inhibition)

References

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