

ENTEROBACTER SAKAZAKII SELECTIVE BROTH (ESSB) ENTEROBACTER SAKAZAKII ISOLATION AGAR (ESIA)

Ready to use liquid medium and isolation agar for the detection of *Enterobacter sakazakii*

PREPARATION

Enterobacter Sakazakii Selective Broth (ESSB): the enrichment broth is ready to use in flasks

Final pH: 6.8 ± 0.2

Enterobacter Sakazakii Isolation Agar (ESIA) the isolation medium is ready to use in plates

Final pH 7.0 ± 0.2

DESCRIPTION

E. sakazakii is a motile peritrichous, gram-negative rod from the family *Enterobacteriaceae*. *E. sakazakii* is an opportunistic pathogen which has been isolated at low levels from powdered infant formula. This organism used to be known as a "yellow pigmented *Enterobacter cloacae*" until 1980, when it was introduced as a new species based on differences in DNA-DNA hybridization, biochemical reactions, and antibiotic susceptibility. *E. sakazakii* is a rare, but life-threatening cause of neonatal meningitis, sepsis, and necrotizing enterocolitis. In general, the reported case-fatality rate varies from 40-80 % among newborns diagnosed with this type of severe infection. The type of meningitis caused by *E. sakazakii* may lead to cerebral abscess or infarction with cyst formation and severe neurologic impairment.

Several outbreaks of *E. sakazakii*, in Neonatal Intensive Care Units (NICUs), have been reported worldwide including countries such as England, Netherlands, Greece, US and Canada. In Canada, two incidents of neonatal meningitis caused by *E. sakazakii* were reported in two Canadian hospitals (1990, 1991). It should be noted that healthy infants may not always be immune to *E. sakazakii* infections. It has been reported that in Iceland, a healthy, full-term, newborn infant became ill prior to hospital discharge and suffered permanent neurological sequelae as a result of an *E. sakazakii* infection.

The method here proposed (ESSB/ESIA) allows to detect *E. sakazaki* in food samples (mainly milk based powdered infant formula products): Enterobacter Sakazakii Selective Broth (ESSB) is a Lauryl Sulfate Broth modification for the selective enrichment of *E. sakazaki*; Enterobacter Sakazakii Isolation Agar (ESIA) is a chromogenic and selective agar medium based on the α -glucosidase reaction, for the isolation and differentiation of *E. sakazaki*

TECHNIQUE

To 25 g of sample add 225 ml of ESSB. Incubate at $37 \pm 1^\circ\text{C}$ for 24 ± 2 hours.

Transfer a loopful of enrichment broth to the surface of ESIA plate and incubate at $44 \pm 1^\circ\text{C}$ for 21 ± 3 hours

Positive result (presumptive): presence of typical blue or blue green colonies

Negative result: absence of typical colonies or presence of violet or mauve coloured colonies

Confirm the typical colonies with the appropriate biochemical test: (production of yellow pigment (+), oxidase (-), L-lysine decarboxylation (-), L-ornithine decarboxylation (+), L-arginine decarboxylation (+), acid from D-sorbitol (-), L-rhamnose (+), D-sucrose (+), D-melibiose (+), amygdalin (+), hydrolysis of citrate (+).

STORAGE

Store the flasks and the plates at $2-8^\circ\text{C}$.

LIMITS

Some coliform bacteria grow on ESIA plates with violet coloured colonies.

REFERENCES

- Simmons, B.P. et al. (1989) *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of powdered infant formula. Infect. Control. Hosp. Epidemiol. 10: 398.
- Van Acker, J et. Al. (2001) Outbreak of necrotizing enterocolitis associated with *E. sakazakii* in powdered milk formula. J.Clin.Microbiol. 39:293-297.

PACKS

5114773

Enterobacter Sakazakii Selective Broth (ESSB)

6x225 ml (ready to use flasks)

541478

Enterobacter Sakazakii Isolation Agar (ESIA) 20

20 ready to use plates

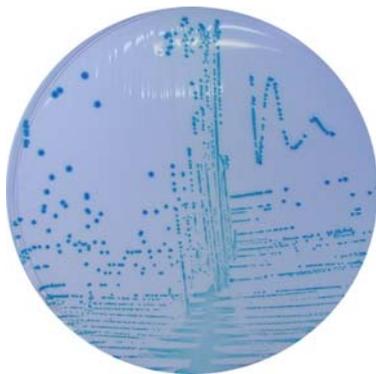
TECHNIQUE

25 g sample + 225 ml ESSB
24 ± 2 hours at 37 ± 1°C

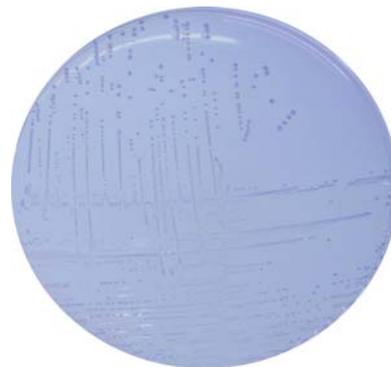
Streak from the cultured ESSB one loopful
on ESIA plate



RESULTS READING



Presumptive positive result
presence of typical blue or blue-
green colonies



Negative Result
Absence of typical colonies or
presence of violet or mauve
coloured colonies

↓

**Confirm *Enterobacter sakazakii*
with biochemical tests**