# SSI ENTERIC MEDIUM

- Detects all Enterobacteria
- Direct identification
- Rapid diagnosis
  - Cost saving



#### S T A T E N S S E R U M I N S T I T U T

prevention and control of infectious diseases and congenital disorders

SSI-Enteric medit

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## SSI-Enteric medium

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#### Design practice

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Statens Serum Institut SSI Diagnostica 2 Herredsvejen 3400 Hillerød Denmark

Tel: +45 3268 8378 Fax: +45 3268 8179 microbiology@ssi.dk www.ssi.dk

#### Introduction

The SSI Enteric Medium is an indicator medium that combines growth differentiating and selective properties without loss of detection. It can be used in routine laboratories for detection of all commonly found enteric pathogens except *Campylobacter*. The medium has been used for more than 20 years in routine laboratories in Denmark.

#### Constituents

- Pancreatic digest of casein Trisodium citrate L-tryptophan Magnesium dichloride Sodium thiosulphate Sodium deoxycholate Sodium glycerophosphate Sodium pyruvate Sodium dodecylbenzenesulphonate
- Yeast extract L-phenylalanine Calcium dichloride Glucose Ferric citrate Neutral red Lactose Agar

#### Preparation

Mix water, medium and 5 N NaOH in the ratio stated on the box. Bring to the boil with frequent agitation and allow simmering gently to dissolve the agar completely. Cool to about 45°C, before dispensing into petri dishes (e.g. 25 ml in 9 cm or 60 ml in 14 cm dishes).

Preparation in an media preparator: Add deionized water, NaOH and medium in the bowl and agitate manually with a whisp. Start the cycle after setting the temperature to 100°C for one minute. Cool to 45°C and pour in petri dishes.

pH 24 hour after production should be 8.1 + - 0.2.

Store petri dishes at 2-8°C and use within 8 weeks. Before use, dry the plates at room temperature for at least 24 hours protected from daylight.

#### Do not autoclave at 121°C!

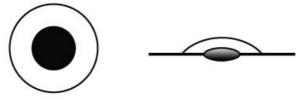
#### Storage and shelf life

Store the dehydrated medium below  $25^{\circ}C$  and use before the expiry date indicated on the label.



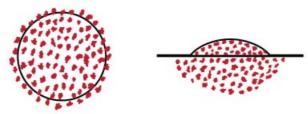
### Quality control

Bacteria	Growth	H <sub>2</sub> S	Phenylalanin	e Lactose	Rough
S. Enteritidis ATCC 13076 P. mirabilis ATCC 12453	+++	+	-	-	-
E. coli ATCC 25922	+++ +++	+	+ -	-+	-
S. sonnei ATCC 25931 E. faecalis ATCC 29212	+++ -	-	-	-	+ -



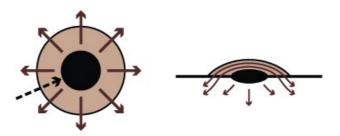
### H<sub>2</sub>S reaction

 $\rm H_2S$  reaction is based on the combined action of sodium thiosulphate, glucose, sodium pyruvate and ferric citrate.  $\rm H_2S$  positive organisms (all with few exceptions are lactose negative) give a distinct ferric sulphide precipitate located centrally and deeply in the anaerobic parts of the colony. The black center is well developed after 20 hours of incubation also in *Salmonella* Typhi.



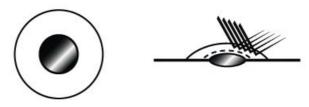
### Lactose fermentation

Lactose fermentation is manifested by acid production and made visible using the indicator combination sodium deoxycholate and neutral red. The acid production is confined to the colony and its immediate surroundings because of the buffering action of deoxycholate resulting in conversion to deoxycholic acid. Deoxycholic acid precipitates and becomes moderately colored by neutral red. The confinement of the reaction to the colony makes recognition of adjacent lactose negative colonies possible.



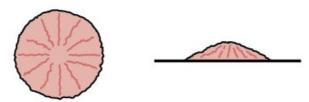
### Phenylalanine deaminase reaction

Phenylalanine deaminase reaction is the result of L-phenylalanin conversion to phenylpyruvate, which with ferric citrate is visible as a brown, diffusible pigment e.g. *Proteus* spp.



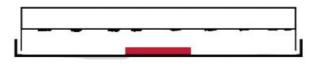
#### Metallic sheen reaction

Metallic sheen reaction presumably depends on a special concentration of calcium deoxycholate on top of the black center in  $H_2S$  producing *Salmonella* (except in *Salmonella* Typhi). The reaction is almost diagnostic for *Salmonella* bacteria.



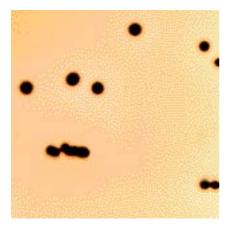
### **Rough transformation**

Rough transformation in *Shigella sonnei* is a well known phenomenon. By adjusting the concentrations of divalent ions (Mg<sup>2</sup>+ and Ca<sup>2</sup>+) to rather high levels, a rough transformation is regularly induced in *Shigella sonnei* affecting the surface and the edge of the colonies. The increased spreading of the colonies makes recognition possible also when located among colonies of other bacteria.



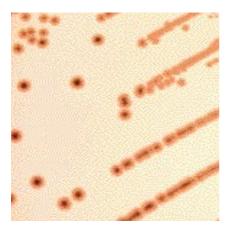
### **Indole reaction**

It is possible to perform the indole reation by placing a piece of filter paper wetted with Ehrlich's paradimethylaminobenzaldehyd reagent in the lid of the inverted petri dish at the time of incubation of the plate. The paper will turn red if indole positive colonies grow on the agar plate. It is important to use filter paper with an acid pH.



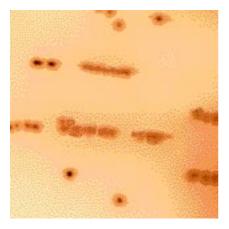
### S. Enteritidis

Typical colonies of zoonotic Salmonella (in this case S. Enteritidis) showing a pale, narrow edge, convex colonies with a black centre ( $H_2S$  production). The metallic sheen reaction is impossible to reproduce on a photo.



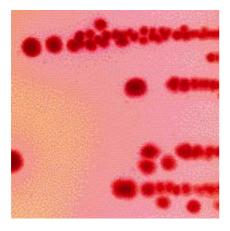
### S. Typhi

Typical colonies of *S*. Typhi. Compared to the zoonotic *Salmonella*, the  $H_2S$  production is much less pronounced, the pale edge is broad, and the colonies are less convex.



### P. vulgaris

A pure culture of *Proteus vulgaris*. The colonies are flat, pale with a dark grey center ( $H_2S$  production). The phenylalanine deaminase reaction is seen as a brown discoloration of the medium below the colonies. Swarming of *Proteus* strains is inhibited. *Proteus* never produce a metallic sheen reaction.



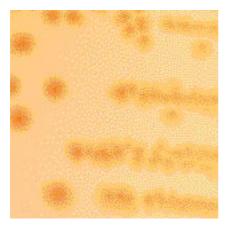
### E. coli

Typical red colonies of a pure culture of lactose fermenting *E. coli*.



### Y. enterocolitica

A pure culture of *Yersinia* enterocolitica. Small, round, convex, pale colonies like "pearls on a string".



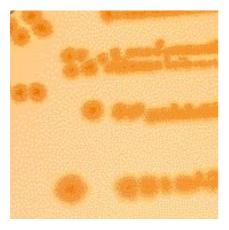
### Sh. sonnei

Large pale rough colonies of *Shigella* sonnei.



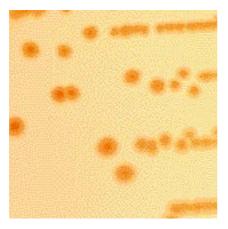
### V. cholera

A pure culture of lactose negative Vibrio cholera, reddish brown translucent round colonies rather flat with curved edges. The translucense is impossible to reproduce on a photo.



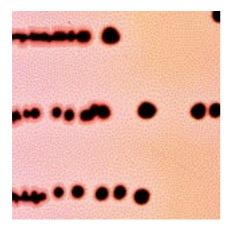
### Aeromonas spp.

A pure culture of lactose negative *Aeromonas* spp., reddish brown opaque round rather flat colonies with curved edges.



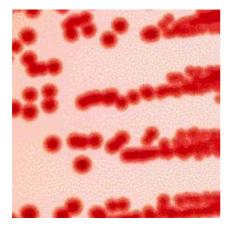
### Plesiomonas spp.

A pure culture of lactose negative *Plesiomonas* spp., reddish brown opaque round rather flat colonies with curved edges.



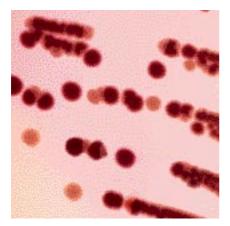
### Citrobacter spp.

H<sub>2</sub>S producing *Citrobacter*. Large, dark grey center and pink edge. *Citrobacter* never produce a metallic sheen reaction.

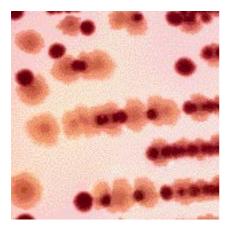


### Klebsiella spp.

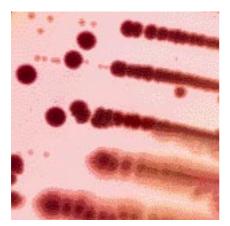
Typical red colonies of a pure culture of lactose fermenting *Klebsiella* spp.



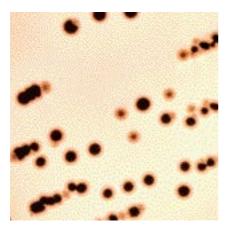
Mixed culture A mixed culture of *E. coli* and *Shigella* spp.



### Mixed culture A mixed culture of *E. coli* and *Shigella sonnei*.



### Mixed culture A mixed culture of *E. coli* and *Yersinia* spp.



### **Mixed culture**

A mixed culture of *Salmonella* Enteritidis and *Salmonella* Typhi.

H <sub>2</sub> S	Lactose	Phenylalanine	Indole	Possible identity
+	-	-	-	Salmonella spp. (incl. arizonae), lactose neg. Citrobacter freundii
+	-	-	+	E. coli (H <sub>2</sub> S pos. variety), Edwardsiella tarda
+	+		-	Citrobacter freundii, lactose pos. Salmonella spp.
+	+	-	+	<i>E. coli</i> (H <sub>2</sub> S pos. variety)
+	-	+	-	Proteus mirabilis
+	-	+	+	Proteus vulgaris
-	-	+	+	Morganella spp., Providencia spp.
-			-	Shigella spp., Yersinia spp., S. Paratyphi A
-	-	-	+	Shigella spp., Vibrio spp., Aeromonas spp., E. coli, Plesiomonas spp.
-	+		-	Klebsiella pneumoniae, Enterobacter spp.
-	+	-	+	E. coli, Aeromonas spp., Klebsiella oxytoca

#### References

1. Blom, M., Meyer, AA., Gerner-Smidt, P., Gaarslev, K. & Espersen, F. 1999 Evaluation of Statens Serum Institut Enteric Medium for Detection of Enteric Pathogens. J. Clin. Microbiol. 37(7): 2312-2316

2. D. Blue-Hnidy, C.M. Kaufman, B. Barrett, S.D. Allen Comparison of the SSI Enteric Medium with commonly used selective and differential media for isolation of Enteric pathogens. Poster C-300, ASM 2004

#### **Available products**

SSI Enteric Medium, 500 g Article No. 34121 SSI Enteric plate, 9 cm Article No. 724 SSI Enteric plate, 14 cm Article No. 22880

#### Additional products for detection of Enteric pathogens

E. coli antisera Salmonella antisera

#### **Ordering and Information**

Statens Serum Institut Diagnostics Sales and Marketing 2 Herredsvejen 3400 Hillerød Denmark Tel.: +45 3268 8378 Fax: +45 3268 8179 microbiology@ssi.dk www.ssi.dk