Evaluation of a new chromogenic Salmonella plating medium

Rolf Reissbrodt[1], Florian Gaull[2], Karsten Fehlhaber[2]

1: Robert Koch-Institut, Burgstraße 37, D-38855 Wernigerode, Germany, Phone: +49-3943-679258, Fax: +49-3943-679207, E-mail: reissbrodtr@rki.de; 2: Universität Leipzig, Institut für Lebensmittelhygiene, An den Tierkliniken 35, D-04103 Leipzig, Germany

Abstract: Oxoid Salmonella Chromogenic Medium (OSCM) was tested for its sensitivity and specificity as well as its use in isolation of *Salmonella* from stool specimens and from porcine faeces. Colonies of *Salmonella enterica* ssp. I, II, IV, and VI-strains grow onto the ivory-coloured turbid plates purple to mauve, size 1-2 mm in diameter. Contrarily, *E. coli*, Enterobacter spp., Klebsiella spp., *S. sonnei* and the most of *C. freundii* strains appear as turquoise to blue colonies. Proteus spp., Providencia spp. and *Morganella morganii* grow light with brownish precipitation. *S. dysenteriae, S. flexneri* and *S. boydii* are widely inhibited. Pseudomonads, Aeromonas spp., grampositive bacteria and *C. albicans* are completely inhibited. Its recovery rate and sensitivity in isolation of salmonella from stool specimens is comparable to Hektoen Enteric agar. OSCM was superior in isolation of *Salmonella* from porcine faeces compared with XLD.

Keywords: *Salmonella* - Isolation - chromogenic medium

Introduction: The widespread occurrence of *Salmonella* spp. requires the need for the rapid detection and identification in feed, food, environment and in clinical samples. Chromogenic *Salmonella* plating media have been developed for the rapid and more reliable identification. These media contain inhibitors for Gram-positive bacteria, the most of nonfermenters and some of the enterobacteriaceae. Oxoid *Salmonella* Chromogenic Medium (OSCM) contain two chromogenic substrates to enable differentiation of *Salmonella* species from other members of the family enterobacteriaceae. Magenta-cap (5-bromo-6-chloro-3-indolylcaprylate) is utilized by *Salmonella* species resulting in purple to mauve colonies. The specificity of C8-esterase (cleaving magenta-cap) is 92 %, the sensitivity 100 %. X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) results in turquoise to blue colony growth in microorganisms that produce the enzyme β-D-galactosidase. This should allow rapid presumptive diagnosis of suspected *Salmonella* spp. with few additional tests only. Furthermore, fastidious Salmonella spp., e.g., *S.* Typhi, *S.* Gallinarum have to grow onto the new chromogenic *Salmonella* plating medium.

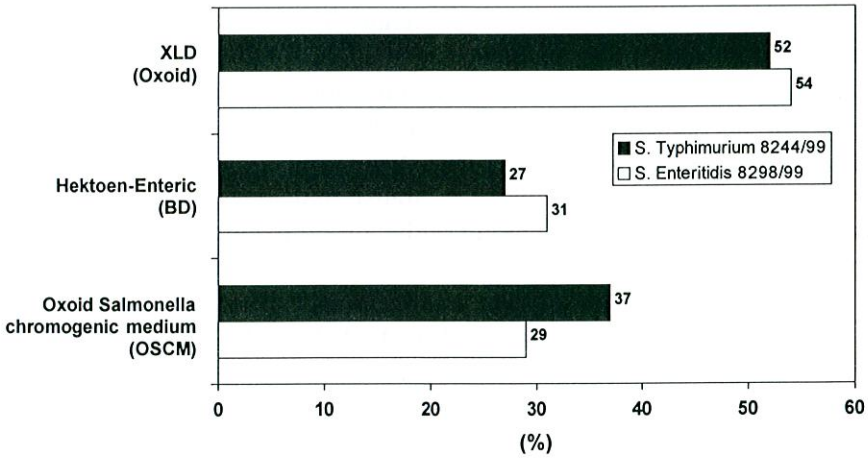
Materials and Methods: Evaluation with pure cultures: *Salmonella* enterica, *Salmonella bongori* and contaminants were freshly cultivated, the grown colonies suspended in phosphate buffered saline (PBS) and diluted to 10^5 to 10^6 cfu/ml for fractionated inoculation. Incubation at 37 °C for 24 hours. Estimation of the recovery rate from the Salmonella plating media: *S.* Enteritidis 8298/99 and *S.* Typhimurium 8244/99 were freshly cultured, the growth suspended and diluted to 10^5 - 10^0 cfu/ml. 0.1 ml of that dilutions were spread over the Salmonella plating media and nutrient agar. Incubation at 37 °C for 24 h. Recovery rate: quotient of counts grown onto the selective plating medium and the counts onto nutrient agar (= 100 %). Recovery of *Salmonella* Enteritidis and *Salmonella* Typhimurium from spiked stool samples using chromogenic and conventional salmonella plating media: Freshly cultivated strains of *S.* Enteritidis 8298/99 and *S.* Typhimurium 8244/99 were suspended in PBS and diluted to the counts/0.1 ml listed in Table 1. 0.1 ml of that suspensions added to 6 ml of selenite broth (Oxoid) containing 1 ml of a mixture of stool and PBS (1:1). Incubated at 37 °C for 20 hours. Subsequently diluted and 0.1 ml of these dilutions spread onto the Salmonella plating media listed. Isolation of Salmonella from porcine faeces: 10 g of porcine faeces, collected rectally with sterile stomacher bags, were pre-enriched in 90 ml BPW at 37 °C for 24 hours. A 0.1 ml aliquot was transferred to 9.9 ml RV broth and incubated at 42 °C for 24 hours. A loopful of the RV broth culture was streaked on XLD agar (Oxoid) and on OSCM agar plates and incubated overnight at 37 °C.

Results: *Salmonella enterica* strains of subsp. I, II, IV, VI and *S. bongori* grew with purple to mauve coloured colonies. *Salmonella* strains of subsp. IIIa and IIIb appear blue-green to lilac. *S.* Typhi, *S.* Paratyphi (B, C), *S.* Gallinarum and *S.* Pullorum grew with small to normal sized colonies. Gram-positive, pseudomonads, aeromonads, *Alcaligenes, Acinetobacter, S. maltophilia* are widely to completely inhibited on all media tested. Strains of the tribe Proteus-Providencia-Morganella are partly inhibited or grew colourless with a brownish precipitate. *Shigella boydii, S. dysenteriae, S. flexneri* formed light, small colonies; *E. coli, S. sonnei, Hafnia alvei* and *S. marcescens* grew with blue-green colonies. Most strains of Citrobacter spp. grew on OSCM with blue-green colonies, some bluish-lilac and few, especially all three *C. amalonaticus* strains tested, similar to Salmonella spp. OSCM behaved in the measure recovery rate similar to Hektoen Enteric Agar (BD). Recovery of Salmonella from spiked stool samples showed the selectivity, specificity and good recognition of Salmonella of OSCM (Table 1). Isolation of Salmonella from porcine faeces underlined this. From 540 tested samples 15 *Salmonella* strains were isolated by use of OSCM and 7 *Salmonella* strains using XLD.

Table 1: Recovery of *S.* Enteritidis 8298/98 and *S.* Typhimurium 8244/98

Cfu inoculated	OSCM (Oxoid)	XLD (Oxoid)	Hektoen Enteric (BD)
***Salmonella* Enteritidis**			
4	no Salm./13 non-Salm.	no growth	no growth
9	14 Salm./4 non-Salm.	no growth	3 Salm.
11	47 Salm./8 non-Salm.	36 Salm.	41 Salm.
***Salmonella* Typhimurium**			
3	54 Salm./6 non-Salm.	9 Salm.	23 Salm.
5	53 Salm./1 non-Salm.	61 Salm.	37 Salm.
11	1 Salm./1 non-Salm.	2 Salm./1 non-Salm.	no growth

Fig. 1: Recovery rate (%) of *S.* Enteritidis 8298/98 and *S.* Typhimurium 8244/98 from various chromogenic and conventional Salmonella plating media



Discussion and conclusion: The Oxoid Salmonella plating medium enabled a good recognition of *Salmonella* spp., in particular of *Salmonella* subsp. I occurring to an extend of ca. 98 % of the isolate. Selectivity and specificity of OSCM should result in improved isolation and presumptive identification of *Salmonella*.