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Evaluation of a new chromogenic medium, **CHROMagar™ Salmonella Plus**, for the detection of *Salmonella* spp. including lactose positive *Salmonella*, *S.* Typhi and *S.* Paratyphi

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Abstract

Infections due to *Salmonella* continue to be a major problem throughout the world. Culture media have several advantages in comparison to PCR and immunological methods : culture media allow antibiotic susceptibility studies, epidemiological studies and, in addition, only living bacteria are detected.

Some recent *Salmonella* chromogenic media are now targeted to the detection of *Salmonella* including lactose positive *Salmonella*, *Salmonella* Typhi, and *Salmonella* Paratyphi. We studied such a new chromogenic medium, designated CHROMagar Salmonella Plus (CHROMagar, Paris, France), in parallel to two other commercially available chromogenic media, focusing the test on the detection of lactose positive *Salmonella*, *Salmonella* Typhi, and *Salmonella* Paratyphi.

All three *Salmonella* chromogenic media demonstrated high level of performance for lactose negative *Salmonella* while CHROMagar Salmonella Plus showed a higher sensitivity for the detection of lactose positive *Salmonella* as now required in food microbiology by the ISO 6579:2002 norm.

Introduction

National *Salmonella* reference centers from many countries continue to collect thousands of *Salmonella* strains every year, showing that infections due to *Salmonella* remain a major health problem.

During the past decades, there was little interest for lactose positive *Salmonella* but a recent concern about them lead to the last revision of ISO 6579 norm in food industry now requiring detection of lactose positive *Salmonella*. Conventional methods and early chromogenic media were not designed for detection of lactose positive *Salmonella*.

In this study, we have tested the ability of three chromogenic commercially available media : SMID2, OSCM and CHROMagar Salmonella Plus, to detect a broad panel of *Salmonella* serovar.

Material and Methods

Culture media. OSCM (Oxoid, UK) and SMID2 (bioMérieux, France) were obtained as prepoured plates. CHROMagar Salmonella Plus (CHROMagar, France) was used as dehydrated culture media and prepared according to manufacturer instructions.

Stock isolates. *Salmonella* strains were provided by the Czech Republic National Reference Center for *Salmonella,* the French National Reference Center for *Salmonella* and the Collège de Bactériologie Virologie et Hygiène des Hôpitaux (France). The lactose characteristic of *Salmonella* strains was determined by Kligler-Hajna Agar/MacConkey Agar. Isolated bacteria were seeded by the quadrant method onto the three chromogenic media.

Reading of plate. Plates were incubated for 24 hours at 37°C. The sensitivity for *Salmonella* detection of the various media was tested: a *Salmonella* strain can be detected if it displays the typical (mauve) colony colour on SMID2, OSCM and CHROMagar Salmonella Plus. Typical colour of colonies of *Salmonella* and other enteric bacteria are indicated on Figure 1.

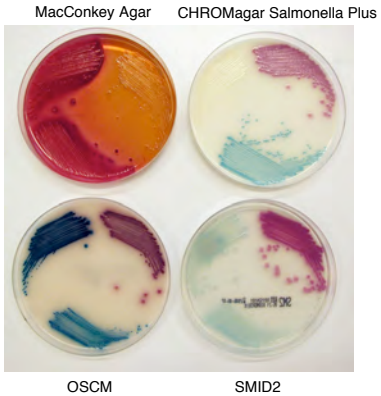

Figure 1. Colony colour of *Salmonella* spp (right top), *E.coli* (left top) and coliforms (bottom) on MacConkey Agar, SMID 2, OSCM and CHROMagar Salmonella Plus.

Results

We first studied 76 ONPG - lactose negative *Salmonella* strains from various serovar (6 *S.*Typhi, 5 *S.*Paratyphi A, 7 *S.*Paratyphi B, 2 *S.*Paratyphi C, 12 *S.*Typhimurium, 5 *S.*Enteritidis, 3 *S.*Brandeburg, 4 *S.*Heidelberg, 3 *S.*Hadar, 2 *S.*Panama, 3 *S.*Kottbus, 2 *S.*Infantis, 2 *S.*Derby, 1 *S.*California, 1 *S.*Goldcoast, 1 *S.*Virchow, 1 *S.*Bredeney, 1 *S.*Mapo, 1 *S.*Indiana, 1 *S.*Saintpaul, 1 *S.*Newport, 1 *S.*Blockley, 1 *S.*Derby) on the three media. The ratio of strains with typical aspect on isolated colonies were respectively for SMID2, OSCM and CHROMagar Salmonella Plus: 72/76 (95%), 75/76 (99%), 75/76 (99%).

We then studied 33 ONPG+ lactose positive *Salmonella* strains on the three media. The colony colour of three ONPG+ *Salmonella* strains is shown on Figure 2.

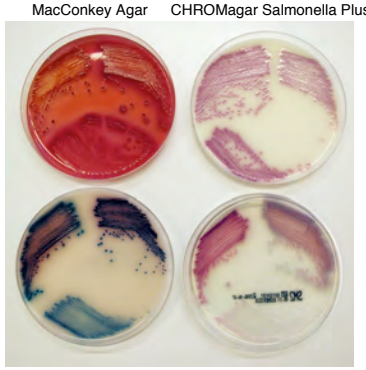

Figure 2. Colony colour of *S.diarizonae* 107/05 (right top), *S.*Worthington AR3910 (left top) and *S.*Senftenberg 7526 (bottom) on MacConkey Agar, SMID 2, OSCM and CHROMagar Salmonella Plus.

On OSCM, isolated colonies from most false negative strains were blue or partially blue. On SMID2, isolated colonies from most false negative strains were colourless or light blue. On CHROMagar Salmonella Plus, isolated colonies from most false negative strains were colourless.

Sensitivities on the three chromogenic media are listed on the following table.

Strains (n) \ Detection on	SMID2	OSCM	CHROMagar Salmonella Plus
S.arizonae (6)	2	0	5
S.diarizonae (13)	7	0	12
S.salamae (3)	1	1	1
S.indica (3)	2	1	3
S.bongori (3)	0	1	1
*S.*Worthington (1)	0	0	1
*S.*Senftenberg (4)	0	0	4
Total (33)	12=36%	3=12%	27=82%

We finally studied 105 faecal samples with CHROMagar Salmonella Plus, in parallel to routine search for *Salmonella*. One positive sample for *Salmonella* was found showing that CHROMagar Salmonella Plus was efficient. In addition this medium seems quite specific since no false positive was detected in this early study.

Conclusion

All three *Salmonella* chromogenic media demonstrated high level of performance for lactose negative *Salmonella* while CHROMagar Salmonella Plus showed a higher sensitivity for the detection of lactose positive *Salmonella* as now required in food microbiology by the ISO 6579:2002 norm.

In addition, preliminary study with clinical samples indicates that CHROMagar Salmonella Plus displays a clear colony contrast from commensal flora.

More investigation on routine detection with CHROMagar Salmonella Plus should be conducted.

Acknowledgments : we thank François Xavier Weill of the French national center reference for *Salmonella* for providing strains.

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