

Earlier detection of *Salmonella* strains using a selective chromogenic medium chromID® *Salmonella* ELITE

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ABSTRACT

chromID® *Salmonella* ELITE, a new selective chromogenic medium was compared to five other media for the detection and presumptive identification of *Salmonella* species isolated from stool samples. This medium contains several substrates, one of them will be hydrolyzed by *Salmonella* species to give mauve colonies. The other bacteria will be blue or colorless. A total of 140 strains were investigated by direct plating or after an enrichment step in Selenite broth for some of them. The sensitivity and specificity were respectively 86% and 95% for chromID® *Salmonella* ELITE and only 78% and 92% for one of the other media. The major advantage is its selectivity level with 42% of inhibited strains compared to other media for which only 25% of non *Salmonella* strains were inhibited. The selectivity is a major parameter mainly for the analysis of stools which contain lots of bacteria which may overgrow *Salmonella* strains. On the basis of these results, chromID® *Salmonella* ELITE can be recommended for the isolation of *Salmonella* strains from stools with and without enrichment in preference to other conventional or chromogenic media.

INTRODUCTION

Salmonellosis remains a major health problem, and its diagnosis most often involves direct detection of bacteria in stools, by primary culture or after enrichment in selective broth. Because of the abundance of enteric bacteria in normal fecal flora, stool cultures on conventional media such as Hektoen, XLD, etc require labor-intensive picking of colonies or give some false positive results. The use of selective chromogenic media reduced the workload with regard to unnecessary examination of suspect colonies, saving time and money.

The aim of this study was to assess the performance of a new chromogenic medium chromID® *Salmonella* ELITE for the early detection of *Salmonella* strains, tested alone or mixed with other bacteria found in human stool.

METHODS

Eighty *Salmonella* strains (30 species) and 60 strains of other bacteria and yeast species coming from human specimens or from a stock collection were cultured onto Hektoen, chromID® *Salmonella* ELITE, chromID *Salmonella*, ASAP (bioMérieux), Brilliance *Salmonella* (Oxoid) and CHROMagar *Salmonella* (BD). One colony coming from a pure culture was suspended in 2 ml of 0.85% aqueous NaCl to get 1×10^8 CFU/ml. Then, 10 µl of this suspension were cultured on each medium. Some 20 mixed culture containing less than 50 CFU of *Salmonella* mixed with 10^4 CFU of *E. coli* per inoculum were also inoculated directly and after enrichment in Selenite F or Rappaport broth. All plates were incubated under ambient atmosphere at 34-38°C. and were read after 18-24 h.

RESULTS

On 80 *Salmonella* strains (Table 1), 74 to 80 strains were able to grow depending on medium type. The less sensitive medium was Brilliance *Salmonella* which lost six strains. On the opposite, the most nutritive media were Hektoen and chromID *Salmonella* which recovered all 80 strains. chromID® *Salmonella* ELITE allowed the growth of 78 strains, so four more strains than Brilliance *Salmonella* and two less than Hektoen or chromID *Salmonella*.

Table 1: Sensitivity results for 80 *Salmonella* strains

| | Hektoen | chromID® <i>Salmonella</i> ELITE | chromID <i>Salmonella</i> | ASAP | Brilliance <i>Salmonella</i> | CHROMagar <i>Salmonella</i> |
|--|---------|----------------------------------|---------------------------|------|------------------------------|-----------------------------|
| Number of <i>Salmonella</i> able to grow | 80 | 78 | 80 | 77 | 74 | 76 |
| True positive | N/A | 69 | 69 | 72 | 67 | 62 |
| Sensitivity after direct plating | N/A | 86% | 86% | 90% | 84% | 78% |

Among *Salmonella* strains able to grow, between 62 to 72 of them gave rise to characteristic colony colors showing sensitivity from 78 to 90%. chromID® *Salmonella* ELITE had a sensitivity of 86%. The highest sensitivity was obtained with ASAP medium which had three additional colored strains but the intensity of color of the colonies was lower on ASAP than on chromID® *Salmonella* ELITE meaning that the result may be subjective with person dependant interpretation. Compared to Brilliance *Salmonella*, chromID® *Salmonella* ELITE recovered two more colored strains. The lowest sensitivity was obtained with CHROMagar *Salmonella* which had 62 colored *Salmonella* strains.



Mixed culture of *S. typhimurium* + *E. cloacae* on chromID® *Salmonella* ELITE agar

Another way to increase the *Salmonella* recovery in stools is to use an enrichment step in a selective medium such as Selenite F or Rappaport broth. In that case, the delay to get a result is longer but it increases the sensitivity of the method. In this study, data in table 2 showed that after an enrichment step in Selenite F broth, chromID® *Salmonella* ELITE was able to recover 19 from the 20 mixed cultures compared to 16 to 18 of the same media. For those 20 mixed cultures, there were only colonies of *Salmonella* on chromID® *Salmonella* ELITE agar vs. only seven to fifteen on the other media.

Table 2: Sensitivity results after Selenite enrichment for 20 *Salmonella* mixed culture

| | Hektoen | chromID® <i>Salmonella</i> ELITE | chromID <i>Salmonella</i> | ASAP | Brilliance <i>Salmonella</i> | CHROMagar <i>Salmonella</i> |
|---|---------|----------------------------------|---------------------------|------|------------------------------|-----------------------------|
| <i>Salmonella</i> True Positive (mauve colonies) | 18 | 19 | 16 | 17 | 16 | 16 |
| Plates with <i>Salmonella</i> pure cultures (selectivity) | 12 | 16 | 7 | 13 | 15 | 12 |
| Sensitivity after enrichment | 90% | 95% | 80% | 85% | 80% | 80% |

Regarding selectivity and specificity results, data are summarized in Table 3 showing that chromID® *Salmonella* ELITE and Brilliance *Salmonella* agar were the more selective media, and were able to inhibit 42% of the non-target strains tested. They were followed by ASAP and then by CHROMagar *Salmonella*. The least selective medium was chromID *Salmonella* with only 15/60 strains inhibited.

Among the non-*Salmonella* strains able to grow, three strains were false positive on chromID® *Salmonella* ELITE, on ASAP and on Brilliance *Salmonella*, and five strains on chromID *Salmonella* and on CHROMagar *Salmonella*. The specificity of each medium ranged from 92 to 95%. chromID® *Salmonella* ELITE, ASAP and Brilliance *Salmonella* were the three more specific agar. The false positive strains were mainly some nonfermentative bacteria which could be easily pre-identified using an oxidase test.

The species inhibited after 18-24 hours were still inhibited after 48 hours. A longer incubation time did not impact the selectivity nor specificity. The only difference was the colony size which was larger after 48 hours.

Table 3: Selectivity and specificity results for 60 non-*Salmonella* strains

| | Hektoen | chromID® <i>Salmonella</i> ELITE | chromID <i>Salmonella</i> | ASAP | Brilliance <i>Salmonella</i> | CHROMagar <i>Salmonella</i> |
|---|---------|----------------------------------|---------------------------|------|------------------------------|-----------------------------|
| Number of non- <i>Salmonella</i> able to grow | 42 | 35 | 45 | 39 | 35 | 42 |
| Selectivity | 30% | 42% | 25% | 35% | 42% | 30% |
| Specificity | 77% | 95% | 92% | 95% | 95% | 92% |

CONCLUSIONS

The present study demonstrates that chromID® *Salmonella* ELITE enables earlier growth and recovery of *Salmonella* even for strains with weak enzymatic activity or those that are lactose-positive. The main advantage of chromID® *Salmonella* ELITE is its higher selectivity against some Gram-negative bacteria such as *E. coli*, which is frequently recovered in stools, and which can overgrow *Salmonella*. Its higher selectivity also decreases false positive results due to the growth of characteristically different colored strains not belonging to *Salmonella*. This will reduce the time to result and the workload for the laboratory. Moreover, the final identification of the species may be performed directly from a colony picked on chromID® *Salmonella* ELITE using VITEK® 2 or VITEK® MS.