Abstract The performance of BBL CHROMagar Salmonella (Becton Dickinson, France), a new selective chromogenic medium for the isolation and presumptive identification of Salmonella spp., was evaluated. On this medium, which is a modification of CHROMagar Salmonella (CHROMagar Microbiology, France) with enhanced selectivity, the colonies of Salmonella are stained in mauve (rose-violet), while those of other organisms appear in blue-green or are not stained by any of the chromogens of the medium. The medium was evaluated with a total of 176 strains of Salmonella and other organisms, consisting of 18 reference strains and 158 clinical isolates. All Salmonella strains except subspecies IIIa and IIIb strains and Salmonella Gallinarum yielded typical mauve colonies. During the evaluation with 107 known positive and 332 unknown stool specimens in a clinical laboratory, a total of 115 and 105 Salmonella isolates were obtained on BBL CHROMagar Salmonella and Hektoen enteric agar, respectively. From the known positive stool specimens, 92 true positive cultures were obtained on BBL CHROMagar Salmonella and 89 on Hektoen enteric agar, yielding sensitivities of 86 and 83%, respectively. From the unknown stool specimens, a total of 27 Salmonella isolates were obtained, with 23 isolated from BBL CHROMagar Salmonella and 16 from Hektoen enteric agar by direct plating (sensitivity 85 and 59%, specificity 99 and 97%, respectively). Seroglutination tests could be performed directly from BBL CHROMagar Salmonella. Compared to conventional isolation media, the time needed for confirmatory biochemical and serological tests was shortened by about 1 day when BBL CHROMagar Salmonella was used. On the basis of these results, the medium can be recommended for the primary isolation and presumptive identification of Salmonella spp. from clinical stool specimens.

Introduction

Many differential selective media for the isolation of Salmonella spp. are available. In most of these, a limited number of biochemical traits (e.g., negative fermentation of lactose; negative lactose and sucrose fermentation; production, by most species and serovars, of hydrogen sulfide; and the ability of species to swarm on semisolid media such as modified semisolid Rappaport-Vassiliadis medium) are used to detect salmonellae not only from human and animal faecal specimens but also from food, milk, and other materials. Most of these media are prone to deliver high rates of false-positive results, mainly due to Proteus and Citrobacter strains from the normal flora mimicking the appearance of Salmonella strains. Some of the media yield high rates of indeterminate isolates, all of which must be differentiated further by biochemical or serological tests before a final result is obtained. Several years ago, more sophisticated media were developed that use a combination of chromogenic substrates and conventional biochemical tests, e.g. Rambach agar (CHROMagar Microbiology, France; Merck, Germany) and SM ID (bioMérieux, Germany). Several evaluations have shown that these two media may be used successfully to subculture organisms from enrichment broth, though neither is very sensitive when used as a primary isolation medium [1, 2, 3, 4, 5].

More recently, a new chromogenic medium for the detection of Salmonella spp., CHROMagar Salmonella (CHROMagar Microbiology, France), has been developed. In a first evaluation, the medium showed good specificity and sensitivity when used for primary isolation and for subculturing from an enrichment medium.
[6]. However, certain other organisms, such as Aeromonas spp., Pseudomonas aeruginosa, and Candida albicans gave false-positive reactions.

In the first part of the present study, the original medium (CHROMagar Salmonella) was modified to increase its selectivity because an overgrowth of the intestinal flora resulted following challenge with low counts of Salmonella in the presence of high counts of certain other members of the family Enterobacteriaceae. Other purposes of the modifications were to increase the stability of the medium and to allow a sufficiently stable industrial preparation of the medium. Test batches of the resulting medium, BBL CHROMagar Salmonella (BCAS), were validated with Salmonella spp. and other organisms from the stock collections of Becton Dickinson, Heidelberg, Germany, and the Robert Koch-Institut, National Reference Centre for Salmonella and other Enteric Organisms, Wernigerode, Germany, to elucidate its productivity and selectivity. In the second part of the study, the use of BCAS was compared with the routine isolation method of a large private laboratory using known positive and unknown human stool specimens.

**Materials and Methods**

**Chromogenic Medium**

The detection of salmonellae on CHROMagar Salmonella (CHROMagar Microbiology) is based on a specific proprietary chromogenic substrate. Salmonella colonies take on a mauve (pink-violent to blue-violet) colouration and are usually surrounded by small mauve halos. Due to the inclusion of two other chromogenic substrates, colonies of organisms other than salmonellae take on a blue-green colouration that may or may not be surrounded by small mauve halos. Organisms that do not metabolise any of the three chromogenic substrates produce transparent, white, or grey colonies.

**Improvement of the Chromogenic Medium**

In the initial tests, the original formulation (which, according to the instructions of CHROMagar Microbiology, shall be suspended in water, boiled briefly, poured into dishes, and used within 1 week) was modified to provide sufficient stability for a ready-to-use plated medium with a shelf life of 9–11 weeks and to increase its selectivity by adding 11 mg/l of novobiocin sodium salt (Serva, Germany), 6.5 mg/l of cefsulodin sodium salt (Takeda, Germany), and 5 mg/l of amphotericin B (Amresco, USA). The antimicrobial agents were prepared as tenfold concentrated solutions in sterile, demineralised water, and 1 ml of each was added to 1 litre of the medium.

**Evaluation and Validation with Pure Cultures**

Altogether, 176 strains from the collections of Becton Dickinson and the Robert Koch-Institut – 92 Salmonella strains (collection strains and clinical isolates) and 84 strains of other organisms (Tables 1 and 2) – were used to evaluate the medium and validate its stability. At Becton Dickinson, plates were inoculated with approximately 10³ cfu/plate for the Salmonella strains and approximately 10⁴ cfu/plate for the non-Salmonella strains from the local collection, using a standard streak for isolation. Plates were incubated aerobically for 20–22 h at 35±2°C and were read independently by two individuals. Growth was evaluated semi-quantitatively as follows: no growth; very weak growth (<50 colonies in the first streak area), weak growth (50–100 colonies in the first streak area only), fair growth (heavy growth in the first streak area and light growth in the second streak area), good growth (heavy growth in the first and second streak areas), and excellent growth (heavy growth in the first, second, and third streak areas). In order to investigate whether the colours were perceived differently by the different readers, colony colours were compared to the Pantone Textile Color Specifier (Pantone, USA).

At the Robert Koch-Institut, plates were inoculated with 10³–10⁴ cfu per plate of the Robert Koch-Institut collection strains and were also streaked for isolation. The plates were incubated for 48 h at 35±2°C and were read after 24 and 48 h. Growth was evaluated using three scores (growth in the first streak area only; growth in the first and second streak areas; growth in the first, second, and third streak areas).

**Evaluation with Normal Stool Specimens Spiked with Salmonellae**

Two stool specimens from healthy individuals were pooled and suspended in normal saline, and the resulting suspension was distributed in 1 ml amounts into sterile tubes. One tube was spiked with 5×10⁵ cfu of Salmonella Typhimurium ATCC 14028 and a second one with 5×10⁵ cfu of Salmonella Enteritidis ATCC 13076. The spiked suspensions were further diluted in tenfold steps in stool suspension. Ten-microlitre portions of the spiked stool suspensions and of the stool suspension not spiked with salmonellae were plated onto BCAS and HEA and were streaked for isolation. The inoculated plates were incubated for 20 h at 35±2°C.

**Evaluation with Clinical Stool Specimens**

BCAS was tested with 439 clinical stool specimens in a large routine laboratory. The standard procedure used for the routine isolation of Salmonella species from stools in this laboratory consists of primary plating onto HEA (Becton Dickinson) and MacConkey II agar (Oxoid, Germany). Additionally, a selenite enrichment broth (Selenite Medium, bioMérieux) is inoculated and subcultured after 18 h of incubation at 36±1°C onto SM ID (bioMérieux) and salmonella-shigella agar (Becton Dickinson). For further identification, the usual procedures, including inoculation of Kliger iron agar and subsequent slide agglutination tests with ovomvalent and polyvalent Salmonella antisera (Behring, Germany), are performed from the growth on Kliger iron agar [7].

For the initial evaluation of BCAS with patient specimens, 107 stool samples previously confirmed as positive by the routine method were collected and refrigerated until the genus was identified. Next, they were streaked onto BCAS and HEA. Cultures were incubated for 20–22 h at 35±2°C. In addition, 332 unknown clinical stool specimens were directly streaked onto MacConkey II agar, HEA, and BCAS. They were incubated for 42–45 h at 35±2°C and read after 24 h and again after 42–45 h. Additionally, a selenite enrichment broth was inoculated and incubated as described above and subcultured onto BCAS, SM ID, and salmonella-shigella agar.

**Identification of Isolates Obtained on the Modified Chromogenic Medium and Hektkon Enteric Agar**

Suspicious isolates on BCAS or HEA (mauve colonies on BCAS and blue-green to blue colonies with or without black centres on HEA) that were recovered from specimens positive for Salmonellosis, as determined by the routine procedure, were further differentiated by means of slide agglutination tests with polyvalent and ovomvalent Salmonella antisera as described above. If a specimen that was found to be negative by the previous routine culture yielded an isolate suspected to be a Salmonella sp., it was tested further by biochemical tests and seroagglutination.

---

**Notes**

- **Salmonella spp.**
- **Pseudomonas aeruginosa**
- **Candida albicans**
- **Enterobacteriaceae**
- **Salmonella Typhimurium**
- **Salmonella Enteritidis**
- **MacConkey II agar**
- **SM ID**
- **Salmonella-shigella agar**
- **Kligler iron agar**
- **Ovomvalent and polyvalent Salmonella antisera**
- **BCAS**
- **HEA**
- **Selenite Medium**
- **bioMérieux**
- **Kligler iron agar**
- **BCAS**
- **HEA**
- **SM ID**