# *Harlequin™ SMAC-BCIG*

***(Sorbitol MacConkey Agar with BCIG)***

**HAL 6**

**Description**
This is a specific substrate medium for the isolation of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS). Pathogenicity of the organism is linked to the production of verocytotoxins (VT1 and VT2), but it should be noted that not all strains of O157 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O103, O111, O113, O145). *E. coli* O157 has been associated epidemiologically with food poisoning outbreaks involving beef burgers and cold cooked meats.

This medium is a modification of Sorbitol MacConkey Agar (SMAC). The addition of the chromogenic substrate BCIG (5-bromo- 4-chloro-3-indoxyl-ß-D-glucuronide) improves the specificty of the medium. *E. coli* O157:H7 is typically sorbitol negative and ? - glucuronidase negative producing pale translucent colonies on this medium. The majority of other *E. coli* strains are ß-glucuronidase positive and sorbitol positive (pink/red colonies). A small percentage of *E. coli* are ß-glucuronidase positive and sorbitol negative and thus appear as blue/green colonies on this medium. Consequently this medium can distinguish between non-O157 sorbitol negative *E. coli* and the genuine toxigenic *E. coli* O157:H7. This reduces the number of unnecessary confirmation tests that are performed. The medium can be made more selective by the addition of Cefixime Tellurite supplement X161 to prepare CT-SMAC. Most workers recommend the use of CT-supplemented medium alongside unsupplemented medium to ensure maximum isolation of *E. coli* O157. This medium can also be useful for the detection of other VTEC producing *E. coli* in conjunction with specifically targetted IMS particles (***Captivate™***).

| **Formula** | **g/litre** |
|---|---|
| Peptone | 20.0 |
| Sorbitol | 10.0 |
| Bile Salts No. 3 | 1.5 |
| Sodium Chloride | 5.0 |
| BCIG | 0.1 |
| Neutral Red | 0.03 |
| Crystal Violet | 0.001 |
| Agar | 12.0 |

**Method for reconstitution**
Weigh 48.6 grams of powder and add to 1 litre of de-ionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C, add 2 vials of X161 CT supplement and pour plates. Dry the surface prior to inoculation.

**Appearance:** Pale red, light violet tinge.

**pH:** 7.1 ± 0.2

Minimum QC organisms: *Escherichia coli O157:H7* NCTC 12900 (non-toxigenic)
*Escherichia coli* NCIMB 50034
*Enterococcus faecalis* NCIMB50030 (inhibition)

**Inoculation:** From O157 Broth LAB 165, surface streak for single colonies.

**Incubation:** 37°C aerobically for 18-24 hr. Examine plates for sorbitol negative, ß-glucuronide negative colonies. Confirm as O157:H7 by serology, (commercial kits or antiserum available).

| **Growth Characteristics** | | | |
|---|---|---|---|
| **organism** | **colony size (mm)** | **shape & surface** | **colour** |
| *E. coli O157:H7*<br>sorbitol -ve<br>ß-glucuronide -ve | 2.5-4.0 | CV.E.G. | Translucent |
| *E. coli* sorbitol +ve<br>ß-glucuronide +ve | 2.5-4.0 | CV.E.G. | Pink/red or purple centre |
| *E. coli* sorbitol –ve<br>ß-glucuronide +ve | 2.5-5.0 | CV.E.G. | Green or translucent with green centre |
| *Note:* Sorbitol positive toxigenic *E. coli* O157:H7 have been isolated and appear as sorbitol positive and ß-glucuronide positive on this medium. To our knowledge these isolates are limited to a small geographical area in Germany. | | | |

**References**
1) Okrend, A.J.G., Rose, B.E., and Lattuada, C.P. (1990) Use of 5-Bromo-4-Chloro-3-Indoxyl- ß-D-Glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J.Food Protection **53** (11) 941-943