

44782 *E. coli* 0157:H7 MUG Agar

Selective agar for the isolation and differentiation of enterohaemorrhagic (EHEC) *E. coli* 0157:H7-strains from food and clinical material.

Composition:

Ingredients	Grams/Litre
Casein peptone	20.0
Meat extract	2.0
Yeast extract	1.0
Sorbitol	10.0
Ammonium ferric citrate	0.5
4-Methylumbelliferyl- β -D-glucuronide	0.1
Sodium chloride	5.0
Sodium thiosulfate	2.0
Bromothymol blue	0.025
Sodium deoxycholate	1.12
Agar	13.0

Final pH 7.4 +/- 0.2 at 37°C

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:

Dissolve 55 g in 1 litre distilled water. Autoclave at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile petri plates. This medium can be made more selective by adding aseptically 0.25 ml of sterile 1% Potassium tellurite solution (Fluka 17774) to 1 litre molten and cooled medium (50°C).

Check the plates under UV light at about 360-370 nm. Slightly blue fluorescence indicates the presence of *E. coli* other than *E. coli* 0157:H7.

Principle and Interpretation:

Currently four different types of intestinal-pathogenic *E. coli* are known: the infant-pathogenic (EPEC), the enterotoxin-forming (ETEC), enterohemorrhagic (EHEC) 0157:H7 *E. coli* strains and the entero-invasive (EIEC) *E. coli* types. Enterohemorrhagic *E. coli* strains have been detected first in hamburgers in the United States. They form toxins which lead to complications in the form of the hemolytic uremic syndrome and thrombotic-thrombocytopenic purpura in 3-20 % of all cases. Thus nowadays the detection of *E. coli* 0157:H7 becomes more and more important for examination of food and clinical material.

Casein peptone, meat extract and yeast extract provide carbon, nitrogen, minerals, vitamins, trace elements and other essential nutrients for growth. Sodium deoxycholate inhibits the growth of most gram-positive microorganisms. Sorbitol together with the pH indicator bromothymol blue enables determination of the ability to degrade sorbitol. March and Ratnam (1) reported the inability of *E. coli* 0157:H7 to ferment sorbitol in contrast to most other *E. coli* strains. Sorbitol-positive microorganisms appear as yellow colonies. Sorbitol-negatives do not change the color and appear as greenish colonies on the green-blue medium. Ammonium ferric citrate and sodium thiosulphate are indicators of H₂S formation. Cultures that produce hydrogen sulfide appear as black-brown colonies due to ferrous sulfide formation and precipitation. *Proteus mirabilis* in particular, displaying biochemical properties similar to those of *E. coli* 0157:H7, can be very easily differentiated from *E. coli* 0157:H7 on account of the brownish discoloration. Subsequently Thomson et al (2) observed the absence of β -D-glucuronidase activity in *E. coli* 0157:H7 in contrast to most other *E. coli*. β -D-glucuronidase cleaves 4-Methylumbelliferyl- β -D-glucuronide to 4-methylumbelliferone and glucuronide. The fluorogen 4-methylumbelliferone can be detected under a long wavelength UV lamp.

Addition of tellurite makes medium much more specific and selective to *E. coli* 0157:H7. Final confirmation and analysis require identification as *E. coli* by biochemical tests and characterization of *E. coli* 0157:H7 and verotoxic properties.

The medium can be inoculated either by pour plate technique or by spreading the sample on the surface of plated medium. Membran filter technique can also be used.

Cultural characteristics after 24 hours at 35-37°C.

Organisms (ATCC)	Growth	Colour of colony	Fluorescence	Sorbitol fermentation
<i>Escherichia coli</i> (25922)	++	yellow	+	+
<i>Escherichia coli</i> 0157:H7	+++	colourless	-	-
<i>Proteus mirabilis</i> (14273)	+++	brown	-	-
<i>Shigella sonnei</i> (11060)	+++	colourless	+	-
<i>Enterobacter aerogenes</i> (13048)	+++	yellow	-	+
<i>Salmonella typhimurium</i> (14028)	+++	yellow with black centre	-	+
<i>Enterococcus faecalis</i> (19433)	-	-	-	-

References:

1. March S.B., and Ratnam S., J. Clin. Microbiol. 23, 869 (1986)
2. Thomposon, J. Clin. Microbiol. 29, 2165 (1990)
3. R.A. Szabo et al., Method to isolate E. coli 0157:H7 from food, J. Food Prot., 10, 768 (1986)