PRO-LAB DIAGNOSTICS

C.T. Supplement

(For the isolation of *E. coli* O157)

FOR IN VITRO DIAGNOSTIC USE

PRODUCT CODE PL.520

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INTENDED USE

Pro-Lab PL.520 C.T. Supplement is to be used in combination with Sorbitol MacConkey Agar to enhance the isolation of *Escherichia coli* O157.

SUMMARY AND EXPLANATION

Escherichia coli serotype 0157:H7 is a verotoxin producing (VT-producing) pathogen. [1,2] This serotype has been reported as an etiological agent in sporadic and outbreak cases of haemorrhagic colitis.[3,4,5] It is also associated with haemolytic uraemic syndrome.[6] Certain *E.coli* serotypes other than 0157:H7 also produce verotoxin. [7,8,9] However, the diarrhoea caused by these other serotypes is not usually bloody. Additionally, *E.coli* serotype 0157:H7 does not ferment sorbitol whereas the majority of other serotypes do ferment sorbitol. [10,11] Therefore, if Sorbitol-MacConkey agar medium is used as a primary screen, the colonies of *E. coli* serotype 0157 appear colourless (non-sorbitol fermenting colonies- NSFC) while colonies of other serotypes appear characteristically pink (sorbitol fermenting colonies-SFC).[11]

Other organisms, such as *Proteu*s and *Morganella,* also grow as NSFC on SMAC medium. In order to inhibit these organisms, as well as sorbitol fermenting organisms (eg. most non-pathogenic *E. coli*) several supplements have been suggested. Cefixime-Rhamnose (CR)-SMAC was introduced in 1991.[12] Cefixime inhibits *Proteus* (NSFC) and rhamnose is fermented by most *E. coli* except serotype O157. The use of potassium tellurite with Cefixime (CT) for the selection of *E. coli* O157 was examined.[13] The use of tellurite in SMAC increased the isolation of *E. coli* O157 by inhibiting other non sorbitol fermenting organisms with no effects on *E. col*i O157. Pro-Lab PL.520 C.T. Supplement is based on this formulation.

DESCRIPTION

Accurate quantities of cefixime and potassium tellurite are lyophilized and provided in individually labelled vials. Each vial is sufficient to supplement 1000 mls of prepared media.

FORMULA

Each vial contains:

Cefixime	0.05 mg
Potassium tellurite	2.50 mg

PROCEDURE

1. To reconstitute each vial of Pro-Lab PL.520, C.T. Supplement add, aseptically, 10 mls of sterile deionized water. After closing the vial, gently agitate to assist reconstitution.
2. Prepare Sorbitol MacConkey Agar according to the manufacturer's instructions, autoclave and cool to 50°C to 55°C.
3. Add the reconstituted contents of one vial of the C.T Supplement to 1000 mls of prepared media. Mix gently and pour into sterile petri dishes.
4. Media may be used immediately. For extended storage at 4°C, eg. up to 7 days, plates should be contained in sealed plastic sleeves or similar packaging.

IN USE

1. Before using selective medium ensure that plates are dry.
2. Inoculate test material onto surface of agar using a sterile inoculating loop or a sterile swab in such a manner as to encourage the growth of isolated colonies.
3. Incubate plates at 37°C for 24 hours.
4. After incubation (no longer than 24 hours), examine plates for small, round, smooth NSF (non-sorbitol fermenting) colonies.
5. Suspect colonies may be tested with the Pro-Lab *E. coli O*157 Latex Test Reagent Kit (PL.070/PL.071). It is an agglutination test kit for the presumptive identification of *E. coli* serogroup O157 antigen on laboratory culture media.

SAFETY PRECAUTIONS

1. Pro-Lab PL.520 C.T. Supplement is offered only as an in vitro material and is in no way intended for a curative or prophylactic purpose.
2. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.

PRESENTATION

Pro-Lab PL.520 C.T. Supplement is supplied 10 vials per box (lyophilized).

STORAGE

Pro-Lab PL.520 C.T. Supplement must be stored at 2°C to 8°C. Kept under these conditions it may be used up to the date of expiry shown on the product label.

REFERENCES

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 = Use by

LOT = Lot number

REF = Catalogue number

 = Manufacturer

EC REP = Authorized Representative in the European Community.

IVD = in vitro diagnostic medical device.

 = Temperature limitation

 = Consult instructions for use.