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(54) Title: DETECTION OF MICROORGANISMS

(57) Abstract: The present invention relates to a medium for the detection of Salmonella, Shigella and E. coli 0157 species, the selective media comprising a growth nutrient base incorporating growth substrates for E. coli 0157 and Shigella, sugar fermentable by E. coli species other than E. coli 0157, bile salts, citrate, magnesium ions and calcium ions in amounts such that the media allows growth of E. coli 0157, Shigella and Salmonella whilst inhibiting growth of other bacteria; an H_2S substrate for detecting hydrogen sulphide production; a chromogenic substrate for detecting β-galactosidase activity; and an indicator substrate for detecting fermentation of the sugar of (ii) and the use of the medium to detect Salmonella, Shigella and E. coli 0157 species in clinical or food or water samples.

DETECTION OF MICROORGANISMS

The present invention relates to the detection of microorganisms.

The present invention provides a medium for the detection of Salmonella Shigella and E. coli 0157 species, the selective media comprising:

a) a growth nutrient base incorporating:
   (i) growth substrates for E. coli 0157 and Shigella;
   (ii) sugar fermentable by E. coli species other than E. coli 0157
   (iii) bile salts, citrate, magnesium ions and calcium ions in amounts such that the media allows growth of E. coli 0157, Shigella and Salmonella whilst inhibiting growth of other bacteria;
b) an H_2S substrate for detecting hydrogen sulphide production;
c) a chromogenic substrate for detecting β-galactosidase activity; and
d) an indicator substrate for detecting fermentation of the sugar of (ii).

The medium according to the present invention is adapted for the selective growth of Salmonella, Shigella and E. coli species (referred to hereinafter for convenience as "target microbes") with inhibition of the growth of other bacterial species. Once sufficiently grown the target microbes may be detected by chromogenic reactions resulting from characteristics of the bacteria and the presence of the chromogenic substrates (b) to (d) in the medium. The medium of the present invention permits rapid microbiological screening of samples for detection of target microbes without requiring inoculation of multiple test media or the performance of additional confirmatory testing, saving both time and money.

The medium is based upon the surprising discovery by the inventors that *E.coli* 0157 has similar resistance to the selective agents normally used to isolate Shigella and Salmonella species which can consist of salts containing deoxycholate, tauracholate, tauraglycocholate or cholateanion (the cation preferably being sodium) and traces of other bile salts either singly or in combination, together with sodium citrate or acetate or phosphate and Calcium and Magnesium ions. Previously these selective agents

were expected to inhibit growth of E. coli 0157. More particularly, the balancing of growth nutrients with bile salts and sodium citrate controls the selective growth of the target microbes, which may then be detected easily by the indicator reactions of sugar fermentation, H_2S production and β-galactosidase activity as detailed below.

The growth nutrient base of the media according to the invention contains all the nutrients required to promote the growth of micro-organisms. Persons skilled in the art will recognise that such nutrients generally include a carbon source, a nitrogen source, a mineral source, a vitamin source and an amino acid source.

Carbon sources that may be included within the growth nutrient base include sorbitol or rhamnose, galactose or IPTG. Other components in the medium may also provide carbon nutrients, such as peptones, which are primarily added as a source of nitrogen.

Nitrogen sources which may be included within the nutrient base include peptones, such as meat peptone, casitone, soy peptones, meat extracts and tryptone.

Sources of vitamins, minerals and amino acids may be included in the nutrient base. While only small quantities of these compounds are required they are essential for maximal growth of the microbes. A readily available source of these compounds is yeast extract. This also contributes metabolisable calcium. Additional calcium may be provided, preferably in the form of calcium chloride. One amino acid which is a preferred ingredient of the selective medium is lysine. Lysine may be provided by the peptones and by addition of the free amino acid.

Other nutrients which may be added to the medium to enhance growth of microbes include potassium ions and sodium chloride (as a source of chloride ions). Calcium and Magnesium ion are critical in influencing the selectivity of the bile salts. Sodium chloride aids in balancing the osmolarity of the medium. Other ions which may be important are sodium ions and carbonate ions (both provided by sodium carbonate), manganese ions, phosphate ions, thiosulphate ions and sulphate ions.

In addition to growth nutrient base components such as carbon, nitrogen, vitamin, mineral and amino acids which may be included in the growth nutrient base medium, the medium of the present invention is characterised by the essential components of (a) (i) to (iii) above.

The pathogens are differentiated using their enzyme characteristics to generate a colour reaction and the selective elements in the media to take advantage of the pathogens increased resistance, over most of the other commensals present in the sample, to ensure the pathogens predominate in the population.

The growth nutrient base also provides bile salts, which together with the sodium citrate and carefully controlled amounts of Ca^{2+} and Mg^{2+} are provided in the medium to inhibit the growth of bacteria other than the target microbes. The bile salts inhibit growth of gram positive bacteria and also inhibit growth of some gram-negative bacteria. Bile salts is intended to be interpreted as a broad term which includes bile salts #3, sodium desoxycholate and ox bile.

The growth nutrient base also provides calcium ions and magnesium ions. These ions are essential ingredients of the growth nutrient base as they provide for optimal growth of E. coli 0157, Shigella and Salmonella species. Preferably Calcium ions are provided as calcium chloride. Magnesium ions are provided as magnesium sulphate.

In a preferred embodiment, a mixture of yeast extract, tryptone, meat peptone, ferric ammonium citrate, sodium carbonate, calcium chloride, sorbitol and magnesium sulphate are used in the nutrient medium, but it is to be understood that these nutrients can be used in combination with other nutrients.

Once the E. coli 0157, Salmonella and Shigella have been allowed to grow selectively on the medium of the present invention characteristic properties of these three bacteria react with the chromogenic substrates to alter the colour of the colonies on the selective media. The presence of the microbe will then be detected. Preferably each

target microbe gives rise to a visually observable colour change which allows detection of that bacteria in a sample containing other bacteria.

As used herein the term "chromogenic" refers to any compound useful in detection systems by its light absorption or emission characteristics. The term is intended to include any enzymatic cleavage products, soluble as well as insoluble, which are detectable either visually or with optical machinery. Included within the meaning of chromogenic substrates are all enzymatic substrates which produce an end product which is detectable as a colour change. Thus a chromogenic substrate may include an enzymatic substrate that will permit the production of detectable colour change upon reaction of an enzyme on the substrate and a substrate which undergoes a specific reaction to produce a colour change. This includes, but is not limited to, any single colour and any combination of these, as well as fluorochromic or fluorogenic compounds which produce colours detectable with fluorescence (e.g. the yellow of fluorescein, the red of rhodamine, and the like). It is intended that other indicators, such as dyes (e.g. pH indicator dyes) and luminogenic compounds be encompassed within this definition.

The colour change may result from the enzymatic cleavage of a chromogenic moiety from the substrate to produce a colour change. As one alternative the colour change may be through formation of a coloured end product by reaction of the chromogenic substrate with a substance synthesised by a particular microbe to produce a coloured end product. As another alternative the chromogenic substrate may undergo a colour change in response to a pH change.

The medium includes three chromogenic substrates, one for detecting hydrogen sulphide production, one for detecting β-galactosidase activity and one for detecting sugar fermentation.

The chromogenic substrate for detecting hydrogen sulphide production allows detection of Salmonella species. Hydrogen sulphide production is characteristic of most Salmonellas species. Examples of preferred chromogenic substrates for the

detection of Salmonella species include ferric compounds which react with hydrogen sulphide produced by Salmonella species to form an insoluble precipitate of ferrous sulphide. Ferrous sulphide is black and thus hydrogen sulphide production by Salmonella may be detected by observing black colonies.

A preferred chromogenic substrate to detect hydrogen sulphide production could be either ferric ammonium citrate or ferric citrate.

The chromogenic substrate for detecting β-galactosidase activity allows detection of E. coli 0157 and some Shigella species. β-galactosidase activity is characteristic of lactose fermenting coliforms and a property held by some Shigella species. Examples of preferred chromogenic substrates for the detection of Shigella and E. coli 0157 include indoxyl-β-D-galactopyranoside, a chromogen which indicates β-galactosidase activity. Beta-galactosidase is an enzyme produced by E. coli 0157 and some Shigella species, and this enzyme reacts with indolyl-β–D-galactopyranoside to produce an insoluble indigo blue precipitate. Other substrates may be used in place of, or in combination with, indolyl-β–D-galactopyranoside, such as 5-bromo-4-chloro-3- indolyl-β–D-galactopyranoside. Other examples of other β-galactosidase substrates, which are chromogenic, include orthonitrophenol-β-D-galactopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside.

In the preferred embodiment, isopropyl-β–D-thiogalactoside is also added to the medium. This ingredient enhances the production of the β-galactosidase enzyme by E. coli 0157 and some Shigella species. Careful addition of this substrate thus improves the sensitivity for the test for these microbes.

The chromogenic substrate for sugar fermentation allows detection of E. coli species other than E. coli 0157 and certain Shigella species. The chromogenic substrate for detecting sugar fermentation is generally provided as a pH indicator such as neutral red, phenol red, or bromo thymol blue along with the carbohydrate. If an organism is capable of fermenting the carbohydrate source the pH of the medium is lowered

providing a colour change, this masks the colour change produced by the indoxyl galactoside. If the carbohydrate is not fermented the pH does not drop and the colour of the colony does not change. The medium contains sugars, which are fermentable by Enterobacteriaceae other than E. coli 0157. The fermentation of such sugars is detected using a pH indicator to give a colour change if the sugar is broken down. Many other pH indicators could be used to demonstrate this reaction.

The initial pH of the medium would be between pH 6.8 and 7.6. After fermentation the colony would have a pH below 5.0

The medium according to the invention is therefore based upon a carefully balanced combination of reactions working together to produce a highly selective and clearly differentiated detection system. Colonies are clearly visible and colour and morphology are the key features. Examples of the results expecting when growing test samples on the medium of the invention are given below.

Most Enterobacteriaceae with the exception of Shigella and E. coli 0157 ferment sorbitol (or similar carbohydrates which could be substituted into this formulation giving the same effect). The presence of Enterobacteriaceae with the exception of Shigella and E. coli 0157 in the test sample will result in fermentation of any sorbitol or similar sugar in the medium. This will result in a pH change which may be detected by a chromogenic pH indicator. If the pH indicator used is phenol red or neutral red the presence of Enterobacteriaceae with the exception of Shigella and E. coli 0157 in the sample will result in red colonies. bromo thymol blue produces yellow colonies. Many other pH indicators could be used to demonstrate this reaction and they may result in a different colour change. Examples of sugars similar to sorbitol which could be used include rhamnose, salicin, inositol, mannitol, dulcitol, d-sorbitol, I-arabinose, I-rhamnose, maltone, d-xylose, trehalose, d-mannose and melibiose and adonitol, or a mixture thereof.

Salmonella will produce hydrogen sulphide. If Salmonella are present in a test sample the hydrogen sulphide produced therefrom will react with the chromogenic

indicator thereof to produce a coloured end product indicating the presence of Salmonella in the test sample. If the chromogenic substrate used is a ferric substrate reaction with hydrogen sulphide will produce ferrous sulphide which is black precipitate. Therefore the presence of Salmonella in a test sample is evidenced by the presence of black colonies on the medium. As Salmonella metabolise the lysine the medium around growing colonies will undergo a pH increase switching on H_2S production. The chromogenic indicator of sugar fermentation will undergo a colour change. If the chromogenic indicator of sugar fermentation is the pH indicator phenol red the presence of Salmonella in a sample will be evidenced by the presence of black colonies with a pink periphery.

Shigella do not ferment sorbitol (or only weakly). Some Shigella species have β-galactosidase activity and some do not. Those with β-galactosidase activity will utilise indoxyl β galactoside to give pale blue colonies. Those which are β-galactosidase negative will give colonies which weakly ferment sugars and thus produce a weak pH change and thus produce a colour change which is dependent upon the pH indicator used. If phenol red is used as a pH indicator pale pink or translucent colonies show the presence of Shigella species which do not have β-galactosidase activity. In the rough phase S. sonnei (the most common pathogen in the United Kingdom), gives a draughtsman-like colony with an umbonate centre which is normally blue and pink or grey periphery. The smooth phase colony is pale blue, round convex entire sometimes with a flattened periphery. S. flexneri will produce a raised entire glossy blue colony whereas β-galactosidase negative strains will give a colourless colony similar to S. boydii and other β-galactosidase negative Shigella.

E. coli 0157 generally does not ferment sorbitol but does produce β-galactosidase giving a blue round entire convex colony. This is the same colour as that of some β-galactosidase producing Shigella and observing this colour would result in a worker being alerted to the fact that they are dealing with either a Shigella spp. or E. coli

0157. Confirmation of either is by a simple slide agglutination followed by a biochemical profile.

Phenylalanine may be added to the medium so that Proteus species produce colonies which makes them easier to distinguish from Shigella with often a brown staining of the medium around the colony. IPTG is added in carefully controlled amounts to ensure that the β-galactosidase reaction is induced but not so much as to provoke too strong a reaction with β-galactosidase positive Shigella.

The medium may optionally include agar, preferably at about 10 – 15 g/l (preferably 11 g/l) or other suitable thickener to gel the medium according to standard gelling techniques for culture media.

Although in most cases the medium will be used as a plating media its use is not limited to such. In addition to standard streak plate methods the medium of the present invention may be formulated so that it may be used with various microbiological testing techniques, e.g. pour plate methods, filtration methods and devices and dip paddles. It is also contemplated that the medium of the present invention be adapted for use in conjunction with film or membrane products such as Petrifilm, whereby the medium of the invention is incorporated into the medium containing an absorbent gel or dry rehydratable film.

The medium according to the present invention may be premixed to yield a powdered medium, i.e. an instant powder mixture, which requires only the addition of water before use.

A specific medium formulation found to exhibit the desired selectivity for the target Enterobacteriaceae whilst allowing Salmonella, Shigella and E. coli 0157 species to be distinguishably identified, wherein the preferred concentration is shown in brackets includes:

INGREDIENT	RANGE grams/litre
Lysine	0.1-1.0 (0.5)
Peptone	2-20 (1.5)
Tryptone	2-20 (1.5)
Yeast extract	1-5 (2.2)
Sodium desoxycholate	0.1-10 (2.8)
Sodium thiosulphate	1-10 (3)
Ferric ammonium citrate or Ferric citrate	0.5-7 (5)
Calcium chloride	0.1-3 (0.7)
Magnesium sulphate	0.01-3 (0.04)
Sorbitol or rhamnose	5-30 (15)
X-β-galactoside	0.005-0.5 (0.075)
IPTG	0.01-0.05 (0.03)
Ox bile	0.1-10 (3.5)
Bile salts N°3	0.1-12 (10)
pH indicator	0.001-0.2 (0.016)
Phenylalanine	0.01-0.2 (0.1)

A range of different species and strains of Salmonella, Shigella and E. coli 0157 have been tested on this media along with a range of other Enterobacteriaceae.

According to the present invention in a second aspect there is provided a method of testing for Shigella and E.coli 0157 comprising:

(a) providing a media according to the first aspect of the invention:

(b) incubating a test sample with the media: and

(c) observing the colour and morphology of microbes grown on the media.

The inventors have demonstrated that E. coli 0157 has similar resistance to the selective agents normally used to isolate Shigella species. Combinations of such agents would, according to the prior art, be expected to inhibit E. coli 0157 and thus growth of microbes according to the above method would previously only have been appreciated as indicating the presence of Shigella species and Salmonellas species and the E. coli 0157 colonies grown on the medium would have been discounted or counted as false positives for Shigella. The realisation by the inventors that Shigella and E. coli share many characteristics allows the two microbes to be detected on the same plate whereas previously this has not been recognised.

Additionally the medium used in the method of the invention further comprises Phenylalanine. This allows additionally the detection of Proteus species.

The test sample may be any clinical sample or a food or water sample. A clinical sample which can be assayed using the method according to the invention can be taken from any part of the human or animal body. Representative clinical samples may be for example faeces, urine, abscess, blood, plasma, serum, bile fluid or amniotic fluid. A test sample may also be any food, environmental or industrial specimen.

Preferably the test sample is incubated at about 35 to 45 °C, preferably at 37 °C. Detectable results can often be obtained after 12 to 16 hours incubation.

Using the test medium according to the present invention to test a liquid food sample E. coli 0157 colonies were green/blue colonies, Shigella sonnei species produced green/blue "fried egg" colonies and sorbitol fermentors were deep pink. If the medium used contained a chromogenic substrate to detect hydrogen sulphide production any Salmonella species in the sample would grow as black colonies with a pink edge. If the medium used contained Phenylalanine any Proteus species in the sample would grow as pale pink colonies with occasionally a brown halo.

The invention will now be described by way of example only in the following non-limiting example.

EXAMPLE

The following medium was dissolved in water and 11 g/l of Agar No. 2 added.

INGREDIENT	**grams/litre**
Lysine	0.5
Meat Peptone	1.5
Yeast extract	2.2
Tryptone	1.5
Sodium desoxycholate	2.8
Sodium thiosulphate	3
Ferric ammonium citrate	5
Sodium carbonate	0.14
Calcium chloride	0.7
Magnesium sulphate	0.04
Sorbitol	15
5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside	0.075
IPTG	0.03
Ox bile	3.5
Bile salts N°3	10
Neutral red	0.016
Phenylalanine	0.1

The medium was plated out and liquid biological samples added and incubated for 16 hours. The following results were observed.

Salmonella results

LAB	Reference	Colony Appearance (morphology. Size in mm)

ID		
S. virchow	A13	Grey & black centre with pink border (CV.E.G. 1.8)
S. kingston	A34	Black centre with pink border (CV.E.G. 2.1)
S. indiana	A35	Grey centre with pink border (CV. E.G. 2.1)
S. karamoja	A51	Grey centre with pink border (CV.E.G.1.9)
S. arizonae	A56	Grey centre with pink border (CV.E.G.2.0)
S. virchow	NCIMB 50077	Grey & black centre with pink border (CV.E.G.2.0)
S. panama	A10	Grey centre with pink border (CV.E.G. 2.5)
S. enteritidis	NCIMB 50073	Grey centre with black border (CV. E.G. 2.5)
S. typhimurium	G77	Black & grey centre with pink border (CV. E.G.2.2)
S. gaminara	A45	Grey & black centre with pink border (CV.E.G. 1.8)
S. kubacha	A46	Grey with pink border (CV. E.G.2.0)
S. albany	A48	Dark grey with pink border (CV. E.G. 2.2)
S. derby	A49	Grey with pink border (CV. E.G. 2.0)
S. assinie	A50	Grey with pink border (CV. E.G. 1.3)
S. enteritidis	A1	Black with pink border (CV. E.G.2.5)
S. enteritidis	A2	Black with pink border (CV. E.G. 1.8)
S. senftenberg	A3	Grey with pink border (CV. E.G. 1.3)
S. senftenberg	A4	Grey with pink border (CV. E.G. 1.3)
S. anatum	A5	Grey with pink border (CV. E.G. 2.0)
S. coeln	A36	Grey with pink border (CV. E.G. 2.0)
S. california	A37	Black with pink (CV. E.G. 1.3)
S. nelolo	A38	Black with grey border (CV.CR.G.2.0-3.0)
S. allandale	A41	Grey with pink border (CV. E.G. 1.5)
S. heidelberg	A42	Grey with pink border (CV. E.G. 2.3)
S. rutgers	A43	Black & grey centre, pink border (CV. E.G. .8)

Shigella results

LAB ID	REF	Colony appearance (morphology. Size in mm)

Shigella sonnei	E57	Blue translucent border (CV.CR.G. 2.5)
Shigella sonnei	E65	Blue translucent border (CV.CR.G. 2.2)
Shigella sonnei	E41	Blue translucent border (CV.CR.G. 2.5)
Shigella sonnei	E63	Blue translucent border (CV.CR.G. 3.5)
Shigella sonnei	E62	Blue translucent border (CV.CR.G. 2.5)
Shigella sonnei	E1	Blue translucent border (CV.CR.G. 2.8)
Shigella sonnei	59	Blue translucent border (CV.CR.G. 2.8)
Shigella sonnei	56	Blue translucent border (CV.CR.G. 3.7)
Shigella sonnei	53	Blue translucent border (CV.CR.G. 2.7)
Shigella boydii	E27	Translucent pink (CV.E.G. 2.0)
Shigella boydii	J82	Translucent pink (CV.E.G. 1.4)
Shigella boydii	J81	Translucent pink (CV.E.G. 2.0)
Shigella boydii	E69	Translucent pink (CV.E.G. 1.3)
Shigella dysenteriae	E64	Translucent pink (CV.E.G. 1.5)
Shigella dysenteriae	E65	Translucent pink (CV.E.G. 2.0)
Shigella flexneri	E36	Translucent pink (CV.E.G. 1.0)
Shigella flexneri	E37	Translucent pink (CV.E.G. 2.0)
Shigella flexneri	E44	Translucent pink (CV.E.G. 0.9)
Shigella flexneri	E47	Translucent pink (CV.E.G. 1.5)
Shigella flexneri	E58	Translucent pink (CV.E.G. 1.5)
Shigella flexneri	E66	Translucent pink (CV.E.G. 2.2)
Shigella flexneri	E67	Translucent pink (CV.E.G. 2.1)
Shigella flexneri	E68	Translucent pink (CV.E.G. 2.0)

E.coli 0157 results

Reference/origin	Colony appearance (Morphology Size in mm)	SMAC Result

A30 agricultural isolate	Green (CV.E.G. 1.0)	+
A32 Barton Hall (pt 2 VTEC 2)	Green (CV.E.G. 2.0)	+
A33 Barton Hall (pt 2 VTEC 2)	Green (CV.E.G. 1.5)	+
A34 Non-toxigenic NCTC 12900	Green (CV.E.G. 1.5)	+
A25 Aberdeen clinical isolate 119 (pt unknown)	Green (CV.E.G. 1.5)	+
A26 Aberdeen clinical isolate C831 (pt 49)	Green (CV.E.G. 1.0)	+
A27 Aberdeen clinical isolate 589 (pt 49)	Pale Green (CV.E.G. 1.5)	+
A28 Aberdeen environmental isolate (pt 31)	Green (CV.E.G. 1.5)	+
A29 Aberdeen minced beef isolate (pt 2)	Green (CV.E.G. 1.5)	+
A35 3169 E. coli 0157:H7	Green (CV.E.G. 1.5)	+
A36 3170 E. coli 0157:117	Green (CV.E.G. 2.0)	+
A37 3171 E. coli 0157:H7	Green (CV.E.G. 2.0)	+
A38 3174 E. coli 0157:H7	Green (CV.E.G. 1.5)	+
A24 C89 Hope	Green (CV.E.G. 1.5)	+
A4 CC97 Preston PHL	Green (CV.E.G. 1.5)	+
A7 CC100 Preston PHL	Green (CV.E.G. 1.0)	+
A8 CC101 Preston PHL	Green (CV.E.G. 1.5	+
A21 C72 Bury General	Green (CV.E.G. 1.5)	+
A22 C87 Hope	Green (CV.E.G. 1.5)	+

Non E. coli 0157 strains

A42 3186 E. coli 014	Pale Green (CV.E.G. 1.5)	+
A20 C71 Hope (sorbitol +, 0157-)	Purple) CV.E.G. 2.0)	-
A44 3191 E. coli 0172	Green (CV.E.G. 2.0)	+
A40 3164 E. coli (sorbitol +, 0157-)	Pale Purple (CV.E.G. 1.5)	-
A39 3198 E hermanii 0157 (sorbitol -, 0157+)	Green (CV.E.G. 1.5)	+
E.coli NCTC 9111	Pale Purple (CV.E.G. 2.3)	-
E.coli NCIMB 50034	Pale Purple (CV.E.G. 2.5)	-

Other organisms tested

Organism	LAB reference Or other	Appearance (morphology. Size in mm)
Enterobacter aerogenes	NCIMB 50029	Purple (CV.E.G. 2.5)
Proteus	1 (unknown species)	Translucent (CV.E.G. 0.7)
Proteus	A (unknown species)	Translucent (CV.E.G. 2.0)
Citrobacter freundii	NCTC 9750	Purple, pale grey centre after 24 h (CV.E.G. 1.5)
Serratia marcescens	ATCC 274	Translucent pink (CV.E.G. 1.5)
Klebsiella aerogenes	1	Purple (mucoid) (CV.E.G. 2.0)
Psuedomanas aeruginosa	ATCC 27853	Translucent orange (CV.E.G. 2.5)
Yersinia enterocolitica	9	Translucent pink (CV.E.G. 0.5)
Enterobacter aerogenes	NCTC 13048	Purple (CV.E.G. 2.5)

CV = CONVEX
E = ENTIRE
G = GLOSSY
CR = CRENATED

CLAIMS:

1. A medium for the detection of Salmonella Shigella and E. coli 0157 species, the media comprising:
   (a) a growth nutrient base incorporating:
      (i) growth substrates for E. coli 0157 and Shigella;
      (ii) sugar fermentable by Enterobacteriaceae other than E. coli 0157
      (iii) bile salts, citrate, magnesium ions and calcium ions in amounts such that the media allows growth of E.coli 0157, Shigella and Salmonella whilst inhibiting growth of other bacteria;
   (b) an H_2S substrate for detecting hydrogen sulphide production;
   (c) a chromogenic substrate for detecting β-galactosidase activity; and
   (d) a indicator substrate for detecting fermentation of the sugar of (ii).

2. A medium according to claim 1 in which the growth nutrient base contains all the nutrients required to promote the growth of micro-organisms.

3. A medium according to claim 2 comprising carbon sources, nitrogen sources, mineral sources, vitamin sources and amino acid sources.

4. A medium according to claim 3 comprising sorbitol or rhamnose, galactose, IPTG as carbon sources.

5. A medium according to claim 3 or 4 comprising peptones as a nitrogen source.

6. A medium according to any one of claims 3 to 5 comprising yeast extract as a source of vitamins, minerals, amino acids magnesium and calcium.

7. A medium according to any preceding claim comprising a balance of bile salts, sodium citrate Calcium and Magnesium ions which allows the target microbes to grow.

8. A medium according to any preceding claim comprising as component (ii) sorbitol or similar sugar.

9. A medium according to claim 8 containing a sugar similar to sorbitol selected from the group containing rhamnose, salicin, inositol, mannitol, dulcitol, d-sorbitol, l-arabinose, l-rhamnose, maltone, d-xylose, trehalose, d-mannose and melibiose and adonitol, or a mixture thereof.

10. A medium according to any preceding claim in which the bile salts are selected from bile salts #3, sodium desoxycholate and ox bile.

11. A medium according to any preceding claim in which the magnesium ions are provided as magnesium sulphate.

12. A medium according to any preceding claim in which the calcium ions are provided as calcium chloride.

13. A medium according to any preceding claim in which the growth nutrient base comprises a mixture of yeast extract, tryptone, meat peptone, ferric ammonium citrate, sodium carbonate, calcium chloride, sorbitol and magnesium sulphate.

14. A medium according to any preceding claim in which the chromogenic indicator of hydrogen sulphide production is a ferric compound which forms a coloured compound in the presence of hydrogen sulphide.

15. A medium according to claim 14 in which the ferric compound is ferric ammonium citrate.

16. A medium according to any preceding claim in which the chromogenic indicator of β-galactosidase activity is selected from indoxyl-β-D-

galactopyranoside, 5-bromo-4-chloro-3- indolyl-β–D-galactopyranoside, orthonitrophenol-β-D-galactopyranoside or 4-methylumbelliferyl-β-D-galactopyranoside.

17. A medium according to any preceding claim in which the chromogenic indicator of sugar fermentation is a pH indicator.

18. A medium according to any proceeding claim further comprising Phenylalanine.

19. A medium according to any preceding claim further comprising agar.

20. A medium according to any preceding claim comprising in grams per litre the following components in the following ranges; lysine 0.1-1.0; peptone 2-20; tryptone 2-20; yeast extract 1-5; sodium desoxycholate 0.1-10; sodium thiosulphate 1-10; ferric ammonium citrate or ferric citrate 0.5-7; calcium chloride 0.1-3; magnesium sulphate 0.01-3; sorbitol or rhamnose 5-30; X-β-galactoside 0.005-0.5; IPTG 0.01-0.05; ox bile 0.1-10; bile salts N°3 0.1-12; pH indicator 0.001-0.2 and Phenylalanine 0.01-0.2.

21. A medium according to claim 20 comprising in grams per litre lysine 0.5; peptone 1.5; tryptone 1.5; yeast extract 2.2; sodium desoxycholate 2.8; sodium thiosulphate 3; ferric ammonium citrate or ferric citrate 5; calcium chloride 0.7; magnesium sulphate 0.04; sorbitol or rhamnose 15; X-β-galactoside 0.075; IPTG 0.03; ox bile 3.5; bile salts N°3 10; neutral red 0.016 and Phenylalanine 0.1.

22. A method of testing for Salmonella, Shigella and E. coli 0157 comprising:
(a) providing a media according claim 1;
(b) incubating a test sample with the media; and
(c) observing the colour and morphology of microbes grown on the media.

23. A method of testing for Shigella and E. coli 0157 comprising:

(a) providing a media according claim 1;

(b) incubating a test sample with the media; and

(c) observing the colour and morphology of microbes grown on the media.

24. A method according to claim 22 or 23 in which the test sample is any clinical sample or a food or water sample.

25. A method according to claim 24 in which the clinical sample may be faeces, urine, abscess, blood, plasma, serum, bile fluid amniotic fluid or other clinical material.