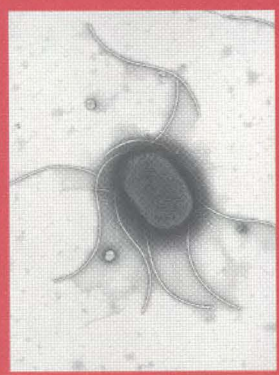
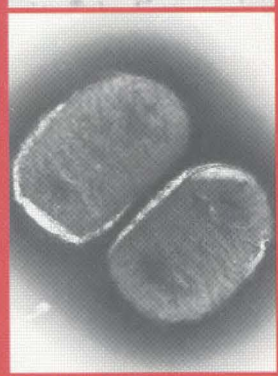
Food-borne Pathogens

MONOGRAPH
NUMBER 5
ESCHERICHIA COLI
SHIGELLA SPECIES





OXOID

SETTING STANDARDS

FOOD-BORNE PATHOGENS
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ESCHERICHIA COLI
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D. E. POST

Technical Support Manager

Price £5.00

March 1998

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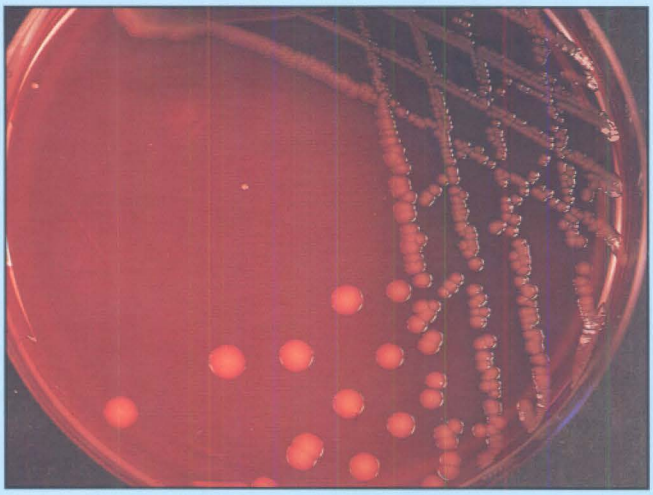
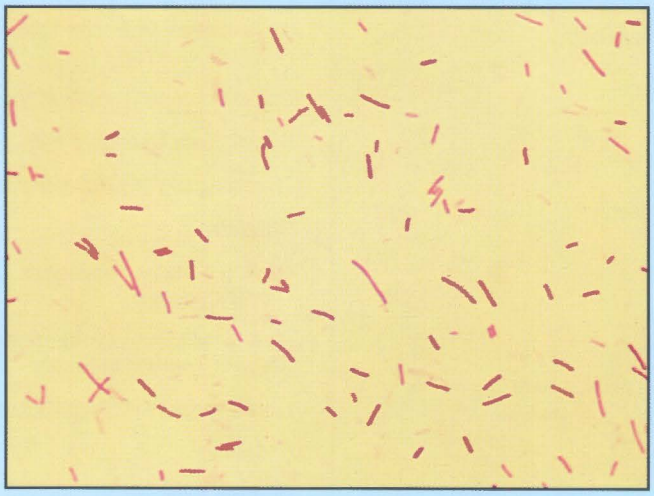
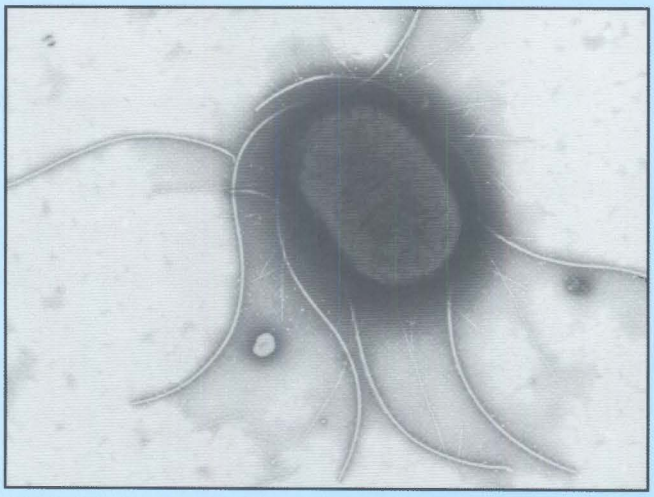
of E. coli 0157:H7 from Foods



Typical appearance of *Escherichia coli* colonies on blood agar.



Gram stain appearance of *E. coli*. *Shigella* species appear similar.
Oxoid Marketing collection.



Electron microscopy appearance of *E. coli* showing flagellae and pili. The pili are the fine, straight projections from the cell.
Photograph: Dr Peter Hawtin, Public Health Laboratory, Southampton, UK.

Escherichia coli and *Shigella Species*

Introduction

Escherichia coli

Escherichia coli (E. coli), originally named *Bacterium coli*, was first isolated in 1885 from the faeces of children.[1] It is the most common aerobic organism in the gastrointestinal tract of man and animals. Because of this, and possession of other attributes desirable in an indicator organism, it has an important role as an index of faecal pollution.

E. coli is also important as a cause of disease in man and animals. Association with animal disease was recognised at the end of the nineteenth century. Recognition of its role in human gastrointestinal disease has been gradual, probably because of the view widely held for many years that the organism is a harmless gut commensal. In man, *E. coli* causes a variety of diseases in contrast to *Shigella species* and *Listeria monocytogenes* which are essentially one-disease pathogens. Pathogenicity of *E. coli* is most commonly recognised because of its frequent occurrence in urinary tract infections and it is also an important cause of pneumonia and meningitis. Realisation that *E. coli* causes childhood enteritis came in the 1940s following an investigation of Summer Diarrhoea involving an infant named Wickens and a pet rabbit named Snowy.[2,3,4]

Further work by the same investigators revealed that over 90% of diarrhoeal infants were infected with a strain subsequently recognised as belonging to serogroup 0111.

Confirmation of the enteropathogenic nature of this serogroup of *E. coli* for young children came from Centers for Disease Control, Atlanta, USA, the UK Central Public Health Laboratory and elsewhere. Subsequently a list began to form of other serogroups recognised as diarrhoeagenic.[3,4] Now it is known that the disease spectrum extends to all ages and ranges from Traveller's Diarrhoea to life-threatening Haemolytic Uraemic Syndrome (HUS). However, until the emergence of *E. coli* 0157:H7, which was first identified as a pathogen in 1982, food industry interest in *E. coli* has largely been restricted to its index organism status.

It should be recognised that enteropathogenic strains of *E. coli* may not entirely conform to the definitions of *E. coli* and faecal coliforms which are applied to their indicator and index organism status. Some may not ferment lactose within 48 hours or do not produce gas. Moreover, the elevated incubation temperatures used in confirmatory tests for faecal coliforms may not permit the growth of enteropathogens.

Diarrhoea-causing strains of *E. coli* are classified into five groups based on the behaviour of the bacterial cells in the presence of the mucosal cells of the host gastrointestinal tract.

E. coli cells may adhere to the mucosa uniformly or in aggregates, may or may not be invasive, may produce haemolysin and one or more toxins including a Shiga-like toxin similar to that produced by *Shigella dysenteriae*, or apparently may not produce toxin at all. These characteristics are used as the basis of the various groupings in which strains are classified. The characteristics shared by strains within the groups and the group names (virotypes) are shown in Table 1.

The different adherence characteristics may be demonstrated on HEp2 tissue culture cells. Adhering strains are divided into subtypes according to the pattern of adherence.[5] Localised adherence (LAEC) strains attach in characteristic localised clusters. Aggregative strains (EaggEC) adhere to epithelial cells, to each other and to glass. Diffuse adherent (DAEC) strains adhere only to HEp2 cells and appear uniformly dispersed in a diffuse pattern over the cell surface.

Placement into the different groupings has been given the name of virotyping. Virotypes correlate well with the type of disease pattern caused, giving information useful in linking isolates with disease symptoms. Observation of the characteristics is also useful in aiding epidemiological investigations. Particular serotypes are associated with virotypes although the correlation is not perfect and some serotypes may occur in more than one virotype.

Serotypes which group into virotypes are shown in Table 2.

Shigella

Organisms of the genus *Shigella* are related to *E. coli* phenotypically and genetically appear the same. However, because existing Shigella names are so familiar it seems likely they will remain in use for the foreseeable future particularly because there are some clear phenotypic distinctions between *E. coli* and *Shigella species* (Table 3). Although most virotypes of *E. coli* cause signs of enteric disease that are different from bacillary dysentery, enteroinvasive (EIEC) strains of *E. coli* cause a very similar condition.

The four species of Shigella, *Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei* have been classified respectively as subgroups A, B, C and D (Table 4). Strains may also be differentiated into serotypes but the number of these is small compared with salmonella and *E. coli*. Biochemical differences between the species (subgroups) are given in Table 5.

All species of Shigella are pathogenic to humans and cause bacillary dysentery of varying severity. Bacillary dysentery, unlike salmonella infection and to an extent *E. coli* infection, is confined to humans and primates. Shigella is still largely ignored by the food industry as a foodborne pathogen but there is an increasing awareness that food may be involved in transmission. There are very few studies of growth and survival of shigellae in foods. The organisms are fairly delicate, but under some circumstances survival may be prolonged. Shigellae are able to grow in foods if refrigeration is poor. Person-to-person and waterborne transmission is of primary importance, especially where standards of hygiene are low.

With the exception of serotype 0157:H7 and other enterohaemorrhagic *E. coli* (EHEC) the low priority given by food microbiologists to *E. coli* and to Shigella as foodborne pathogens means that little effort has yet been put into optimising methods and culture media for use to detect these pathogens in foods. Most detection methods for *E. coli* are concerned with finding it as an organism indicating faecal pollution and, until better ones are developed, these methods have to be used in the search for pathogenic strains, supplemented by methods derived from clinical microbiology.

In this publication, discussion about media and methodology available for detection of *E. coli* will largely be in the context of the indicator and clinical situations. For 0157:H7 and other EHEC serotypes there will be a greater emphasis on examination of foods because rapid progress has been made in developing methods specifically for this group of pathogenic strains.

The media and methods for *Shigella spp.* are almost entirely based on clinical practise; the validity for use with foods is still largely unknown. A method specifically intended for testing foods is published in the FDA Bacteriological Analytical Manual but it is clear that much further work has to be done to determine whether the method is optimal. An outline of this method is given on page 54.

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1 Escherich, T. (1885) Die Darmbakterien des Neugeborenen und Saüglings. *Fortschritt der Medizin* **III,** 231–236.
2 Bray, J. (1945) *J. Pathol.* **57,** 239–247.
3 Bray, J. and Beaven, T.E.D. (1948) *J. Pathol.* **60,** 395–401.
4 Bray, J. (1973) *Arch. Dis. Child.* **48,** 923–926.
5 Gonzalez, R., Diaz, C. and Marino, M. *et al.* (1997) *J. Clin. Microbiol.* **35,** 1103–1107.

Occurrence of *Escherichia coli* and *Shigella Species* in Foods

E. coli is present in large numbers amongst the normal flora of the gastrointestinal tract of man and other animals. For this reason it is readily found in the environment with consequent constant opportunity for transmission to food raw materials and finished products. However, because the organism is generally a harmless gut commensal the enteropathogenic potential of a strain must be established before pathogenic significance is assigned to any isolate. This is a consideration separate from the significance of isolating *E. coli* when using it as an index organism for possible presence of unrelated enteric pathogens or as an indicator organism of hygienic standards.

E. coli behaviour in foods is similar to that of Salmonella and other Enterobacteriaceae.

The temperature for growth ranges from 7°C to 48°C with an optimum of 35–37°C. Enterotoxigenic strains appear as a group to be able to thrive better than non-toxigenic strains at lower temperatures. At the other end of the temperature range, the maximum for *E. coli* 0157 is lower than for other *E. coli*. Strains may not be able to grow at 44°C, a temperature commonly used for isolation of *E. coli*.

E. coli will grow over a pH range of 4.5 to 9.0 but the ability to grow at a particular pH is influenced by other parameters including the nature of the acidulant, water activity (aw) and temperature.

Under ideal conditions the minimum aw for growth is 0.95.

There is evidence that *E. coli* is more resistant than Salmonella to curing agents.

E. coli generally competes more effectively than Salmonella with spoilage organisms but even with its greater capability for growing at lower temperatures it is likely to be overgrown by psychotrophic organisms such as Pseudomonas.

Presumptive evidence of probable pathogenicity may be obtained from determination of the serotype because there is close correlation between some serotypes and their ability to cause disease in man.

The ubiquity of *E. coli* makes it likely that it may be present in a very wide variety of foods including some that are markedly acid. The organism is relatively robust, which is one of the characteristics which makes it so useful as an index and indicator organism. *E. coli* may frequently be found on food plants irrigated with waste water. Fruit and vegetables may also be directly contaminated with animal faeces. *E. coli* 0157:H7 infection has been caused through consumption of apple juice because of the organism's ability to survive the acidity of the juice. The original source of contamination was probably contact in the orchard between fallen apples and cattle faeces. Serotype 0157 is strongly associated with cattle and outbreaks of disease in humans are frequently linked to inadequately cooked beefburgers and milk that has not been pasteurised or has become contaminated post-pasteurisation. Experimentation has shown that contaminated salami, despite its low water activity and other preservation measures involved in its production, could constitute a hazard for up to a month if seriously contaminated.

Whilst ground beef products have been implicated in most 0157:H7 outbreaks, other meats including turkey and ham have been associated, generally when they are made up in sandwiches. Cross contamination during sandwich preparation appears likely to be the cause. Person-to-person spread has been reported between hospitalised patients and health care staff and this appears to support the view that *E. coli* 0157 could also be transmitted by food manufacturing staff if their hands are lightly soiled. There is still some doubt whether cattle are the sole source of *E. coli* 0157. Reports of isolation from pork, lamb and venison suggests that pigs, sheep and deer may also be carriers. Experimentally, chicks have been colonised by small numbers of this serotype indicating that poultry may also be a source. More recently *E. coli* 0157 has been isolated from goat faeces, suggesting that this animal too may be a source of infection.

Because of the serious health consequences of infection by *E. coli* 0157:H7 and other enterohaemorrhagic serotypes, the transmission of this virotype has received the most intensive study. Other virotypes are probably spread by broadly similar vehicles and the association of poor quality drinking water with Traveller's Diarrhoea is well known and has led to a folk-lore all its own, generally evoking the vengeful activities of rulers in exotic locations.

The use of proper hygienic practices when handling foods of animal origin and proper heating of foods during cooking, must remain an important defence against *E. coli* enteric infections. The importance of water in transmission dictates that it must be of high hygienic quality whether used in food preparation or for drinking.

Shigella

Shigella infection is primarily restricted to higher primates and man, therefore cattle, poultry, sheep and pigs do not constitute the serious infection sources that they may be for *E. coli*. Although shigellosis is generally waterborne or transmitted person-to-person via the faecal–oral route it may also be foodborne. The most usual means of foodborne spread amongst humans is by food handlers with poor personal hygiene. Transmission by flies is also important in the spread of Shigella. Shigella is not heat-resistant and the foods incriminated are generally those consumed uncooked and those that have undergone a final preparation following initial cooking. Most often incriminated have been raw vegetables, potato salad and shellfish.

Shigellae are less robust than *E. coli* but even so are able to survive a variety of environmental conditions for significant periods of time. There is some variability in the susceptibility of the different groups to particular conditions, but information is incomplete because studies have mostly been limited to *Sh. sonnei* and *Sh. flexneri*.

There is a general capacity to survive cold storage and freezing. Growth is inhibited below 6°C and above 45°C but the effect of temperature is much influenced by pH and concentrations of sodium chloride and nitrite.

Heat resistance is not a feature of *Shigella spp*; death occurs rapidly at about 65°C.

There is little resistance too, to low pH, pH values less than 4.0 cause rapid death.

Shigellae die only slowly under low water activity conditions, a characteristic which is probably responsible for their success in surviving on surfaces and in dried foods.

In summary, shigellae appear able to survive well at subzero and refrigeration temperature in a variety of foods and in conditions that occur commonly during food handling and preparation.

Pathogenic Properties of *E. coli* Virotypes

Enterohaemorrhagic *E. coli* (EHEC)

The most frequently encountered serotype in this virotype group is *E. coli* 0157:H7 which causes disease ranging from mild diarrhoea to haemorrhagic colitis. Disease will progress in some patients to Haemolytic Uraemic Syndrome (HUS) with its associated renal failure. Other EHEC serotypes may also cause HUS.

A toxin is produced that is virtually identical to Shiga toxin (verocytotoxin, VT). It may be a contributory cause of the intense inflammation that occurs with EHEC infections.

All age groups are affected but most commonly children and the elderly. Cattle appear to be the major source of 0157:H7.

EHEC strains attach to small intestine mucosal cells and change their surface structure by a process called attachment and effacement in which the finger-like projections (microvilli) on the mucosal surface become longer where the bacteria are in proximity but not attached to the mucosa and are lost where the bacteria are attached. EHEC strains invade mucosal cells but, following invasion, do not spread to adjacent cells. A Shiga toxin-producing strain of *E. coli* 0111 which lacks the attaching-effacing gene and which shows enteroaggregative adherence properties has been reported.[1] This strain also possesses a gene for heat-stable toxin and consequently shows a combination of virulence factors usually associated separately with two virotypes.

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* strains resemble *Vibrio cholerae* in that they adhere to the mucosal surface of the intestine but do not invade. Two toxins are produced that cause diarrhoea by their action on the mucosal cells. There is little inflammation. Vomiting and fever accompany the diarrhoea. ETEC infection can be fatal, especially in children. Infection by ETEC strains in adults frequently occurs where sanitary conditions are poor and is commonly called Traveller's Diarrhoea.

Enteroaggregative *E. coli* (EAggEC)

Enteroaggregative *E. coli* cause a persistent diarrhoea in children. They resemble ETEC strains in that they bind to small intestine mucosal cells but differ in not adhering uniformly but in small clumps (Aggregates). EAggEC strains are not invasive and produce two toxins. This group is only recently recognised and its pathogenic significance is still relatively unknown.

Enteropathogenic *E. coli* (EPEC)

EPEC strains also do not attach uniformly to mucosal cells but the appearance is different from that of EAggEC strains. Like EHEC strains, EPEC strains attach to and efface mucosal cells. Invasion of the cells occurs and there is inflammation. Production of toxins is not a feature of EPEC strains although some strains have been reported to produce heat-labile toxin (LT) and heat-stable toxin (ST). More recently production by some strains of cytolethal distending toxin (CLDT) has been recognised. Amongst some of these strains simultaneous production of CLDT and ST was observed and in other work the simultaneous production of VT and LT with CLDT has been seen. Diarrhoea is probably caused by alteration of the cell signalling systems following invasion.

EPEC strains cause severe, often fatal, diarrhoea in children. In adults, infection is a cause of Traveller's Diarrhoea.

Enteroinvasive *E. coli* (EIEC)

EIEC strains cause a disease that is indistinguishable from Shigella dysentery but they do not produce a Shiga-like toxin. The organisms actively invade cells of the colon and spread to adjacent cells.

A diagrammatic representation of the appearance of *E. coli* cell attachment for the different virotypes is shown in Table 1.

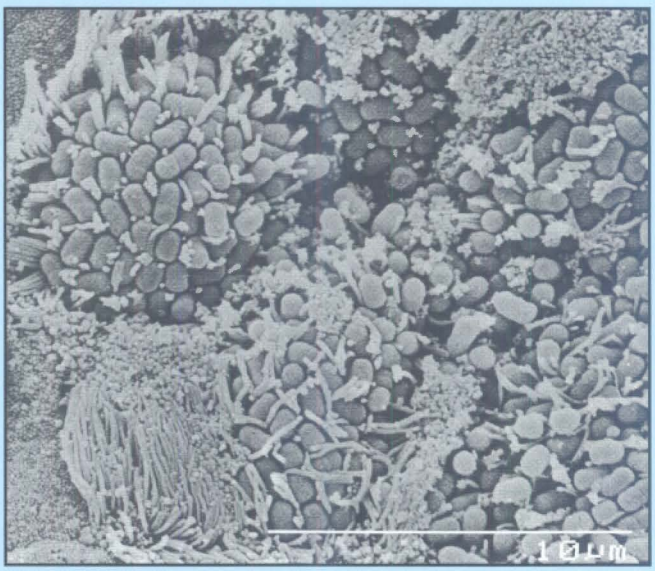
Serotypes that are contained in the virotype groups are listed in Table 2.

Diffuse-adherent *E. coli* (DAEC)

DAEC strains uniformly cover the surface of Hep2 and Hela cells. They do not produce heat-labile or heat-stable toxins or Shiga toxin and do not invade epithelial cells. These strains have been associated with mild diarrhoea without blood or faecal leucocytes in children in Central America.

Reference

1 Caprioli, A. (1997) Cited in a review of a meeting of the European Study Group on Enterohaemorrhagic *Escherichia coli*. *Clinical Microbiology and Infection* **3,** 391.



Attachment of enteropathogenic (EPEC) *E. coli* cells to the small intestinal mucosa.

Photograph supplied by Dr Stuart Knutton, Institute of Child Health, University of Birmingham, UK.

Table 1 – Properties of virotypes.

Virotypes	Adhesion and Invasion Characters	Toxins	Disease Symptoms
Enterotoxigenic (ETEC)*	 Adhere uniformly but do not invade.	Heat-labile (LT). Heat-stable (ST). LT is similar to cholera toxin and acts on mucosal cells.	Cholera-like diarrhoea but generally less severe.
Enteropathogenic (EPEC)	 Adhere in clumps. Invade host cells, attach and efface.	Not apparent.	Infantile diarrhoea, vomiting.
Enteroinvasive (EIEC)**	 Invade cells of colon. Spread laterally to adjacent cells.	No Shiga-like toxin has been detected.	Cell-to-cell spread and disease is similar to dysentery.
Enterohaemorrhagic (EHEC)	 Adhere tightly. Attach and efface host cells. Invade.	Verocytotoxic Shiga-like toxin.	Bloody diarrhoea. Haemorrhagic colitis. May progress to Haemolytic Uraemic Syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP).
Enteroaggregative (EAggEC)	 Adhere in clumps but do not invade.	ST-like toxin. Haemolysin. Verocytotoxin reported in some strains.	Diarrhoea. Some strains have been reported to cause Haemolytic Uraemic Syndrome (HUS).

*See also Diffuse Adherent *E. coli* (DAEC) page 5; **EIEC show some serological relatedness to *Shigella spp.*

Table 2 – Some serotypes of *E. coli* contained in virotype groupings.

Virotype	Serotypes
Enterotoxigenic (ETEC)	0 6, 0 8, 0 15, 0 20, 0 25, 0 27, 0 63, 0 78, 0 80, 0 85, 0 115, 0 128, 0 139, 0 148, 0 153, 0 159, 0 167.
Enteroaggregative (EAggEC)	The enteroaggregative phenotype is found in a wide range of serotypes. It is not known yet whether all are human pathogens. A recent report has linked serogroups 062, 073 and 0134 with outbreaks of diarrhoeal disease.†
Enteropathogenic (EPEC)	0 18, 0 44, 0 55, 0 86, 0 111*, 0 112, 0 114, 0 119, 0 125, 0 126, 0 127, 0 128, 0 142.
Enterohaemorrhagic (EHEC)	0 6, 0 26, 0 46, 0 48, 0 91, 0 98, 0 111*, 0 112, 0 146, 0 157, 0 165.
Enteroinvasive (EIEC)	0 124, 0 143, 0 152.

*Note serotype 0111 may be enteropathogenic or enterohaemorrhagic.

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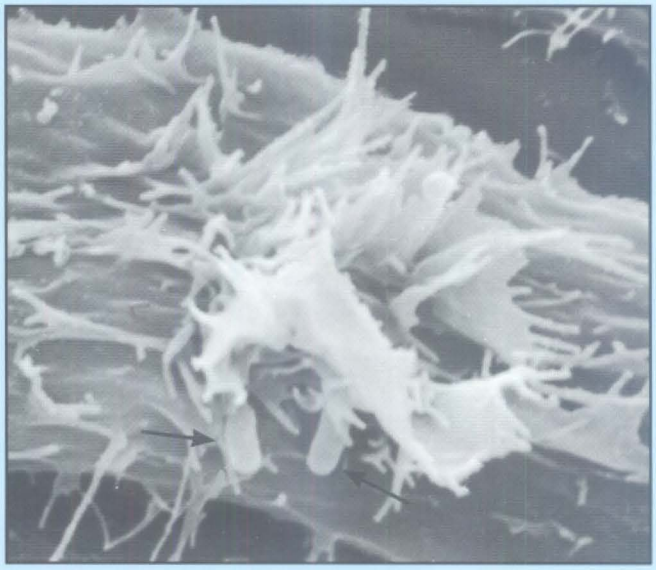
Recent advances in verocytotoxin-producing *Escherichia coli* infections. International congress series 1072. Karmali, A.M. and Goglio, A.G. (eds). Elsevier 1994. Making Safe Food. Harrigan, W.F. and Park, R.W.A. (1991). Academic Press Limited, London.

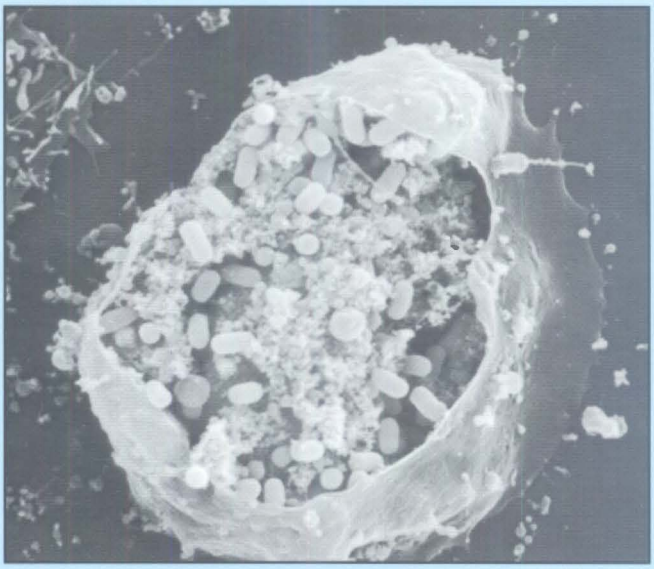
Dr Bernard Rowe, personal communication.

†Smith, H.R., Cheasty, T. and Rowe, B. (1997) *The Lancet* **350,** 814–815.



Invasion of cultured epithelial cells by enteroinvasive (EIEC) *E. coli*. EIEC strains cause ruffling of the host cell surface and are taken up by the cell during this process. Two bacteria (arrowed) are visible within the ruffles.

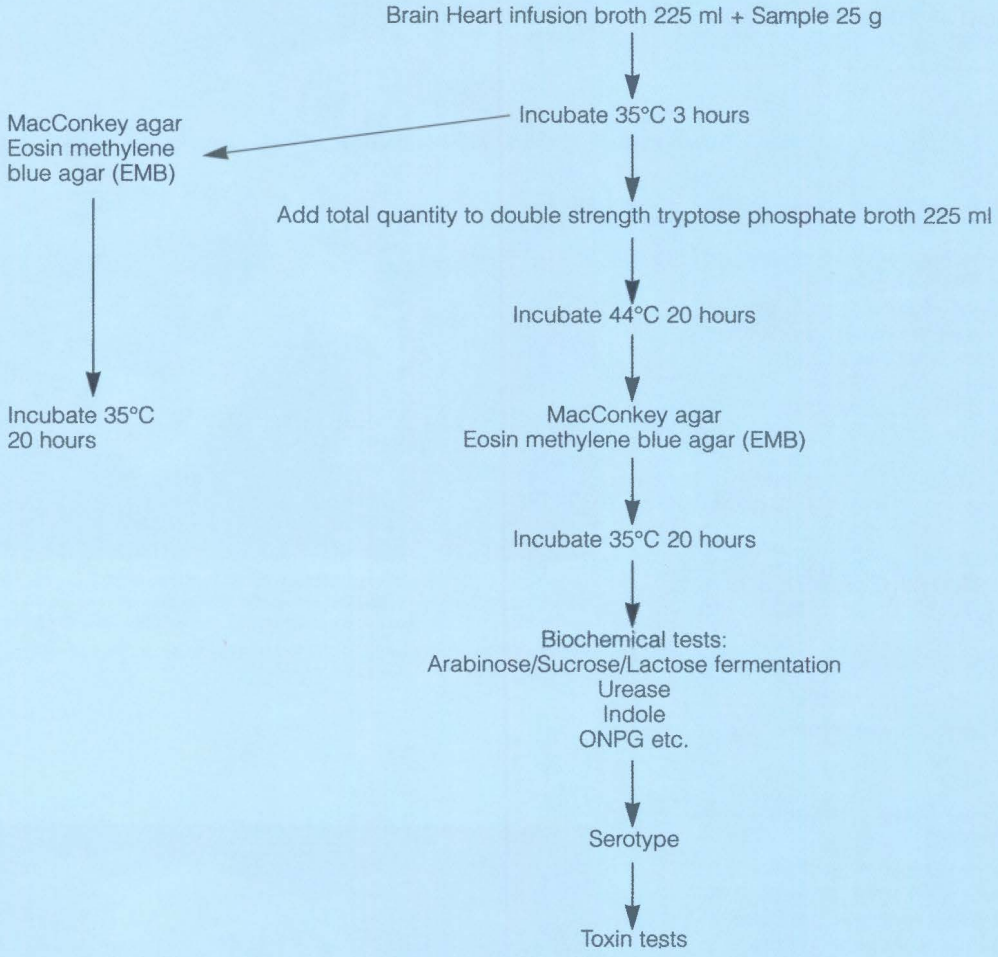
Photograph supplied by Dr Stuart Knutton, Institute of Child Health, University of Birmingham, UK.



Cell lysis caused by intracellular growth of *E. coli*. Lysis releases bacteria which then infect other cells.

Photograph supplied by Dr Stuart Knutton, Institute of Child Health, University of Birmingham, UK.

FDA-BAM Method for Isolation and Identification of Enterovirulent *E. coli* (EEC)*



This is an abbreviated version of the procedures used. Microbiologists intending to follow this method should consult the FDA Bacteriological Analytical Manual for full details.

Reference

FDA Bacteriological Analytical Manual 8th Edition 1995.
Chapter 4. AOAC International, Arlington Va.

*Methods for detection of Enterohaemorrhagic *E. coli* are given on pages 42 and 46.

Liquid Media for Detection of *E. coli*

Liquid media for *E. coli* are almost all directed towards the detection of *E. coli* and coliforms as organisms indicating faecal contamination and general hygiene conditions. *E. coli* is generally differentiated from coliforms in secondary tests designed to show whether organisms that have grown at 35–37°C are capable of growth and production of indole, and also, gas from lactose at 44°C.

The use of *E. coli* as an index organism to signal the possibility that ecologically similar pathogens may also be present appears to have been suggested first by Schardinger. Shortly afterwards Theobald Smith prompted the beginning of the use of coliforms for this purpose when he pointed out that since *E. coli* is so generally found in the intestinal contents of man and animals its presence outside the intestines may be taken as evidence of faecal contamination.

Broth media for *E. coli* are generally used in the Most Probable Number (MPN) technique when searching for the organism as an index and indicator organism in water and in milk. It is only recently, with the emergence of enterohaemorrhagic strains of *E. coli* as a cause of serious disease, that broth media have been formulated specifically for the purpose of selective enrichment. Very little similar development has yet occurred for the other virotypes.

Early in the twentieth century MacConkey[1] formulated his medium for the detection of lactose-fermenting, bile-tolerant organisms and for many decades the broth bearing his name has been a standard medium for the primary isolation of coliform bacteria. In the original medium litmus was employed as an indicator of acid production from lactose. This was subsequently replaced by neutral red as a more satisfactory alternative. Later, Childs and Allen[2] showed that neutral red is variable and may sometimes prevent *E. coli* from growing. Bromocresol purple is less inhibitory and was substituted for neutral red. This replacement has not been complete and both indicators are still in use. However, MacConkey broth containing bromocresol purple is generally preferred because it provides a stronger and more sensitive indication of acid production by its very marked colour change from purple to yellow.

The expense of bile and its innate variability led Jameson and Emberley[3] to experiment with replacing bile with an anionic detergent. Teepol was chosen and used at a concentration of 0.1%. When manufacture of Teepol ceased it was replaced satisfactorily with sodium lauryl sulphate and used with the same Oxoid basal medium.[4] By this time the use of membrane filtration had become established and the first report of lauryl sulphate broth described it for this methodology.

Considerable advantages of standardisation of culture media performance can be gained by the use of chemically defined media. A defined medium based on glutamic acid was first advocated by Folpmers.[5] His ideas were tested by Burman and Oliver[6] who consequently introduced some modifications. Their conclusions were confirmed in a comparison of MacConkey broth and glutamic acid medium carried out by the Public Health Laboratory Service.[7]

Glutamic acid medium containing glucose gave full agreement with MacConkey broth in 24 hours but close inspection of the results showed MacConkey broth to have various performance defects.

More work was carried out on the glutamic acid medium[8,9,10,11] resulting in further improvement and a version with detailed formulation changes.[12] Oxoid Minerals Modified Glutamate Medium (CM607 + LI24) is based on Gray's 1964 medium.[11]

With the addition of agar it is used as the resuscitation medium in the technique for the detection of *E. coli* in foods using tryptone bile agar.[13]

A different route in the development of media for detecting *E. coli* and coliforms in water has been taken in America. There, lauryl sulphate broth (lauryl tryptone lactose broth) is commonly used and is currently specified for this purpose by the American Public Health Association. It is also used as a medium for confirming the presence of *E. coli* in foods.[14] The medium may also be used in the control of ice cream and monitoring of dairy hygiene.[15] A variation of the medium in which mannitol replaces lactose has been described to facilitate detection of non-lactose-fermenting strains of *E. coli.*[16]

Boric acid has been used in lactose broth to confer selectivity for *E. coli*[17] but appears to have gained little acceptance.

The necessity to subculture and incubate at 44°C to confirm the presence of *E. coli* in mixed coliform cultures by its production of gas and indole has led to the formulation of media in which both of these characteristics can be demonstrated in a single tube. Schubert[18] described a non-inhibitory medium for this purpose. It was later used as the basis of another medium which gave improved detection of *E. coli* in mixed culture,[19] and yet another medium which performed similarly but was easier to prepare.[20]

The dye brilliant green is used in conjunction with bile in brilliant green-bile broth, a medium first described in the 1920s for detection and confirmation of members of the coli-aerogenes group.[21] Success with this formulation is very dependent on careful balance of the inhibitors brilliant green and bile in order to inhibit growth of *Bacillus* and *Clostridium* species which otherwise would cause false positive reactions by fermentation of lactose to produce gas. This medium is used in the food industry to detect *E. coli*, the coli-aerogenes group and psychotrophic coliform organisms when incubated at 44°C, 32°C and 4°C respectively.

The entire Enterobacteriaceae family can be used as indicator organisms in the evaluation of processed foods. Mossel and his colleagues substituted glucose for lactose in their EE (Enterobacteriaceae Enrichment) broth and overcame discrepancies that can arise from atypical enterobacteria in lactose-containing coli-aerogenes media. EE broth is used as an enrichment medium following pre-enrichment in tryptose soya broth in the examination of foods and animal feeds. Incubation at 44°C favours growth of *E. coli* and similarly thermotrophic bacteria.

The emergence of *E. coli* 0157:H7 and other enterohaemorrhagic serotypes as causes of severe enteric disease necessitated the development of media for enrichment of these organisms to assist their detection in foods.

Prior to this it was usual to select *E. coli*, generally by incubation at elevated temperatures, from growth in media that supported the entire coli-aerogenes group. The pathogenic potential of the *E. coli* isolated would then be established by appropriate tests.

The need to detect *E. coli* 0157:H7 in clinical, food and environmental samples required enrichment media which are sensitive and preferentially select enterohaemorrhagic strains from other *E. coli*.

In response to this need, Doyle and Schoeni[23] modified tryptone soya broth by adding phosphate buffer and made it selective using bile salts and novobiocin. The medium was used in a hydrophobic grid-membrane-filter-immunoblot procedure developed specifically for the isolation of *E. coli* 0157:H7 from foods. The medium was subsequently modified to create one which enabled better expression of antigens recognised by a monoclonal antibody in an ELISA technique for rapid detection of *E. coli* 0157.[24] Casamino acids were added and acriflavine hydrochloride replaced novobiocin which had been observed to have a suppressive effect on production of the antigen. Other workers also employed modified tryptone soya broth in an improved rapid technique for isolation of *E. coli* 0157 from foods.[25] Their initial experiments using selective buffered peptone water showed that cefixime at 0.05 mg/litre was inhibitory to a strain of *E. coli* 0157. However, growth of this strain in a modified tryptone soya broth containing concentrations of vancomycin, cefixime and cefsulodin present in their selective buffered peptone water was nearly as vigorous as that in buffered peptone water without antibiotics.

Chapman and his colleagues[26] also used buffered peptone water made into a selective enrichment broth by adding vancomycin, cefixime and cefsulodin. The medium was used in a study of cattle as a possible source of *E. coli* 0157 infections in man. This medium and variations on selective tryptone broth are currently specified in Public Health Laboratory Service methodology.[27]

Doyle and Schoeni[23] used selective tryptone soya agar and Padhye and Doyle,[24] sorbitol MacConkey agar containing 4-methyl'-umbelliferone- β-D glucuronide (MUG) as plating media for isolation of *E. coli* 0157 from their enrichment cultures. Chapman and his Public Health Laboratory Service colleagues use sorbitol MacConkey agar containing potassium tellurite and cefixime as selective agents.

Buffered peptone water modified by the addition of casamino acids, yeast extract and lactose is used in a technique for screening samples of beef. Samples are enriched for 10 hours in the medium and then the broth is membrane-filtered. Fluorescence antibody staining is then applied to the membrane and cells detected by epifluorescence microscopy.[28]

The same modification of buffered peptone water has been employed in a 5-hour screening procedure for detecting *E. coli* 0157:H7 in beef.[29] The organism is detected using an antibody-direct epifluorescent filter technique (Ab-DEFT) and confirmation of the results is obtained in 24 hours by capturing *E. coli* 0157 cells on immunomagnetic beads which are then plated on sorbitol MacConkey agar. Speed of growth in the buffered peptone water enrichment is accelerated by pre-warming the medium before inoculation and incubation in a shaking water bath.

E. coli (EC) broth is a Standard Methods medium for differentiating and enumerating faecal and non-faecal coliform organisms. It was developed from an earlier buffered lactose broth by Perry and Hajna[30] who substituted tryptose for peptone and added bile salts number 3 to inhibit Gram-positive organisms. Growth of *E. coli* was enhanced. A selective medium[31] made from EC broth by the addition of novobiocin has been reported as superior to both buffered tryptone soya broth containing bile salts and novobiocin (EHEC enrichment broth EEB)[23] and tryptone soya broth containing vancomycin, cefsulodin and cefixime in a procedure which uses immunomagnetic beads for concentration of *E. coli* 0157.[32] Immunomagnetic separation of serotype 0157 from selective enrichment cultures is now an established procedure.[33]

In yet another development, Universal Pre-enrichment Medium supplemented with Oxyrase™ has been used successfully for simultaneous recovery of heat-injured *E. coli* 0157:H7 and *Yersinia enterocolitica* cells in foods.[34] Subculture into a selective broth is necessary to assist isolation from mixed flora.

Despite recognition of pathogenic *E. coli* groups other than verocytotoxigenic strains, there has been little attempt to formulate culture media to aid their recovery. Mehlman and Romero[35] investigated the effectiveness of standard *E. coli* isolation procedures for recovery of serotypes associated with human gastrointestinal illness and concluded that traditional *E. coli* media may not be suitable for enrichment; glucose should be present for use by slow lactose fermenters and various growth factors are necessary for nutritionally fastidious strains. On the evidence from their work they developed two media; tryptone-phosphate (TP) and glucose-lactose-Tween 80 (GL80) intended for use following initial repair of injured cells in brain heart infusion. Formulae are given on page 18.

Clearly much still has to be done if media that select the pathogenic from the non-pathogenic serotypes are to come into existence.

One further development in this direction has been the formulation of a single-tube screening medium for *E. coli* 0157:H7 which contains sorbitol and flagella antigen H7 antiserum.[36] In this medium organisms are presumptively identified as *E. coli* 0157:H7 by their failure to ferment sorbitol, and immobilisation in the semi-solid medium because of the reaction of their flagella with the antiserum. The effectiveness of this approach to detection is obviously reduced if a strain is non-motile but, as the authors remark, the results suggest that media may be formulated in a similar way for detecting other specific serotypes of *E. coli.*

A number of the media mentioned in this review are available from Oxoid and are described in the following pages. Formulae are also given for manufacture of some selective enrichment media.

In recent years the development of substrates which are cleaved by enzymes specific to particular microorganisms to give coloured hydrolysis products has introduced a valuable new tool for increasing the rapidity and accuracy of presumptive identification. Coliform organisms produce β-galactosidase and this activity may be detected by the ONPG test in which the compound ortho-nitrophenyl- β-D galactopyranoside (ONPG) is hydrolysed by the enzyme to give a yellow final product. 95–100% of *E. coli* strains (but not serotype 0157) also possess the enzyme β-glucuronidase. This characteristic is shared with some strains of Shigella, Salmonella and Yersinia but no other enteric organisms. Consequently detection of this enzyme in an isolate confers on the isolate a high probability that it is *E. coli.* A variety of substrates are now available that give fluorescent or coloured end products that presumptively identify strains as *E. coli* by fluorescence or colony colour.[37] The fluorogen 4-methylumbelliferyl- β-D glucuronate (MUG) was one of the earliest substrates used. It has been replaced to some extent with later more satisfactory compounds, including 5-bromo-4-chloro-3-indolyl- β-D-glucuronate (BCIG), which give coloured rather than fluorescent end products and do not possess some disadvantages associated with MUG.

Chromogenic substrates are now routinely used in liquid

media and simultaneous detection of coliforms and *E. coli* is possible by detection of β-galactosidase and β-glucuronidase. The disadvantages associated with MUG are generally less apparent in liquid media and it may be incorporated successfully in a variety of formulations including selective media used in the MPN technique.[38] Oxoid MUG Supplement BR71 is described on page 38.

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MacConkey Broth

MacConkey Broth
Code: CM5

A differential medium containing neutral red for the detection of coliform organisms in water and milk examination.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075

pH 7.4 ± 0.2

Directions

To prepare single strength broth, add 40 g to 1 litre of distilled water. Mix well and distribute into containers fitted with fermentation (Durham) tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Description

For many years, MacConkey Broth has been the standard medium for the detection of coliform bacteria, and has been recommended for this purpose by the Public Health Laboratory Service Water Committee[1] and the World Health Organisation.[2] The Oxoid product conforms to their specification for water testing and also to the formulation specified by the Department of Health[3] for milk grading.

The advantages of MacConkey Broth in the presumptive coliform test are the low proportion of false positive reactions (PHLS Water Subcommittee[4]) and the fact that most strains of *Escherichia coli* produce a positive reaction within 24 hours.[5] Disadvantages, due to variability of the peptone and bile salts contained in the original medium, have been overcome by large scale production, pooling of batches and careful quality control – including titrimetric standardisation of the bile salts by a method described by Burman.[6]

The neutral red is pre-tested for the absence of toxic substances before inclusion in the Oxoid medium. Childs and Allen[7] showed that some samples of neutral red were inhibitory. For those who prefer it, this medium is also available with bromocresol purple as the indicator – for details of this alternative medium and the presumptive coliform test see MacConkey Broth Purple CM5a.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Escherichia coli (turbidity + gas) ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

The neutral red indicator is carefully selected for this formulation and therefore shows no inhibitory effect. However, the more sensitive reaction of bromocresol purple in MacConkey Broth (Purple) CM5a is often preferred.

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MacConkey Broth (Purple)

MacConkey Broth (Purple)
Code: CM5a (Powder)
Code: CM6a (Tablets)

A differential medium containing Bromocresol purple for the detection of coliform organisms in water and milk examination.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Bromocresol purple	0.02

pH 7.2 ± 0.2

Directions

Powder To prepare single strength broth add 40 g to 1 litre of distilled water. Distribute into containers fitted with fermentation Durham tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Tablets Add 1 tablet to 10 ml of distilled water. Insert a fermentation (Durham) tube and sterilise by autoclaving at 121°C for 15 minutes.

Description

MacConkey Broth has long been used as a presumptive medium for the detection of the coli-aerogenes organisms. In the original medium, litmus was employed as the indicator of acid production but, in later publications, MacConkey suggested neutral red as a more satisfactory alternative. Childs and Allen[1] showed that some samples of neutral red exerted an inhibitory effect on the growth of *Escherichia coli* in this medium.

Bromocresol purple is less inhibitory, and the colour change from purple to yellow provides a more sensitive and definite indication of acid formation; therefore this indicator is used in Oxoid MacConkey Broth-Purple which corresponds to the alternative formulation recommended in "International Standards for Drinking Water".[2]

Technique

The presumptive coliform examination consists of the inoculation of measured volumes of water into tubes of MacConkey Broth-Purple which are incubated at 35°C for 48 hours. Choice of volumes for inoculation will depend on the bacteriological grade of the water being tested; for "medium" waters the Public Health Laboratory Service Water Committee (1961) recommend one 50 ml, five 10 ml and five 1 ml quantities of water – 50 ml and 10 ml amounts being added to their own volume of double-strength MacConkey Broth while the 1 ml amounts are each added to 5 ml of single-strength MacConkey Broth. Acid formation is indicated by a yellow colouration of the broth, and gas formation is indicated by an amount of gas at least sufficient to fill the concavity at the top of the Durham tube. From the number of tubes showing the presence of acid and gas, the most probable number of (presumed) coliform bacteria present in 100 ml of the original water may be estimated by reference to probability tables available in many publications dealing with this subject. For the differential coliform test, each MacConkey tube showing acid and gas is then subcultured into a fresh tube of MacConkey Broth and incubated at 44°C. Formation of gas within 48 hours is practically specific for *Escherichia coli* and indicative of faecal pollution of the original water sample.

MacConkey Broth-Purple is also suitable for the bacteriological examination of milk, as described by Davis.[3] This method, which is basically similar to that used for the examination of water, consisting of the inoculation of suitable dilutions of the milk into tubes of this medium, followed by incubation and inspection, was originally recommended by the Department of Health, London.[4]

MUG Reagent BR71 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR71 to this medium will enhance the detection of *Escherichia coli*. See MUG Reagent BR71, page 38.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Quality Control

Positive control:
Escherichia coli (acid + gas) ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

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Minerals Modified Medium

Minerals Modified Medium (Sodium Glutamate – L124)
Code: CM607

Formula

(double strength)	grams/litre
Lactose	20.0
Sodium formate	0.5
L-cystine	0.04
L(–)aspartic acid	0.048
L(+)arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate $7H_2O$	0.200
Ferric ammonium citrate	0.020
Calcium chloride $2H_2O$	0.020
Dipotassium hydrogen phosphate	1.80
Bromocresol purple	0.020
pH 6.7 ± 0.1	

Directions

Double Strength Dissolve 5 grams of ammonium chloride in 1 litre of distilled water. To this add 22.7 grams of Minerals Modified Medium Base CM607, and 12.7 grams of sodium glutamate L124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Single Strength Dissolve 2.5 grams of ammonium chloride in 1 litre of distilled water. To this add 11.4 grams of Minerals Modified Medium Base CM607, and 6.4 grams of sodium glutamate L124. Mix to dissolve completely. Sterilise by autoclaving for 10 minutes at 116°C; alternatively heat to 100°C for 30 minutes on three successive days.

Note

To improve the stability of the dehydrated medium on storage the sodium glutamate L124 is supplied separately and must be added to the basal medium CM607.

The pH of the final medium is critical for optimum performance and the sterilised broth should be checked to confirm that it is at pH 6.7 before use.

Differences in heating procedures cause differences in final pH value. If necessary the heating procedure should be adjusted so that the final pH, after sterilisation, is 6.7.[1]

Description

A chemically defined medium based on glutamic acid was first advocated by Folpmers[2] for the enumeration of the coliform group of bacteria in water.

The Public Health Laboratory Service[3] carried out a trial and concluded that glutamic acid media containing glucose gave too many false positives in 48 hours. Gray[4] modified a glutamate medium containing lactose and later published a formulation for an improved Formate Lactose Glutamate Medium.[5]

This latter medium was incorporated in another large trial carried out by the PHLS[6] in which three glutamate media were compared with Teepol Broth (Jameson and Emberley[7]) and MacConkey Broth. The results showed that Gray's improved formate lactose glutamate medium was superior to the other glutamate media on trial.

The report carried criticism of the mineral content of the medium and it was considered that it could be improved by modifying the amounts of minerals.

A co-operative investigation was carried out between the London Metropolitan Water Board Laboratories and Oxoid Laboratories which resulted in a Minerals Modified Glutamate Medium CM289.

The Oxoid Minerals Modified Glutamate Medium was used in further PHLS[6] trials and the results with the Oxoid medium confirmed the superior performance of glutamate media reported previously (PHLS[6]).

The superior performance of Minerals Modified Glutamate Medium over MacConkey Broth is due mainly to improved detection of *Escherichia coli*. Table 3 (adapted from PHLS[8]) illustrates the results obtained in the trial.

The Table shows that for chlorinated water, incubation for >18 hours is required for glutamate media to demonstrate their superiority.

The medium and method are fully described in Her Majesty's Stationery Office Report 71.[1]

More recently further trials showed Minerals Modified Glutamate Medium to be the medium of choice for the detection of *Esch. coli* in chlorinated waters, especially where the numbers of organisms concerned were small.

It was also found superior to Lauryl Tryptose Lactose Broth for detection of small numbers of *Esch. coli* in other water, although the latter medium gave quicker results (18–24 hours compared to the 48 hours required by Minerals Modified Glutamate Medium).

Papadakis[10] investigated the isolation of *Esch. coli* from sea-water and found Minerals Modified Glutamate Medium to be better than MacConkey Broth formulations. However, to avoid high salt concentrations in the broth he recommended 1 ml only of sea-water to be added to 10 ml of single-strength MMG medium. Higher volumes of sea-water must be diluted out 1/10 with MMG medium.

Technique

The technique known as the Multiple Tube Method, Dilution Method or the Most Probable Number (MPN) Method is used with Minerals Modified Glutamate Medium. A trial comparing membrane filtration and multiple tube methods showed glutamate medium to be unsatisfactory for use with membranes for enumerating coliform organisms in water.[11]

With waters expected to be of good quality, the medium should be inoculated with one 50 ml volume and five 10 ml volumes. With waters of more doubtful quality, five 1 ml volumes should be used in addition to the 50 ml and 10 ml volumes. Dilutions of the 1 ml volumes may be required for polluted water and the 50 ml volume may be omitted.

The larger volumes of water (10 ml and 50 ml) are added to equal volumes of double-strength medium, whereas the 1 ml volumes (or dilutions of them) are added to 5 ml of single-strength medium.

The tubes are incubated at 35°C and examined after 18–24 hours. All those tubes showing acid (yellow colour in the medium) and gas in the inverted inner (Durham) tube should be regarded as "presumptive positive" tubes, including those in which gas appears after tapping the tube. The tube may only have a bubble of gas after tapping. The remaining tubes should be re-incubated and examined after another 24 hours. Any further tubes becoming "positive" should be treated as "presumptive positives".

Each "presumptive positive" tube should be sub-cultured to a tube of Brilliant Green Bile (2%) Broth CM31 and incubated for 24 hours at 44°C.

Table 3 – Comparison of Minerals Modified Glutamate Medium and MacConkey Broth by numbers of positive tubes.

	Number of tubes yielding								
	False positive reactions			Coliform organisms			*Esch. coli*		
	18 hr	24 hr	48 hr	18 hr	24 hr	48 hr	18 hr	24 hr	48 hr
Unchlorinated samples									
MacConkey Broth	17	37	100	625	806	1060	467	528	582
Minerals Modified Glutamate Medium	2	20	97	557	858	1175	503	707	764
Chlorinated samples									
MacConkey Broth	4	19	49	125	216	315	77	121	128
Minerals Modified Glutamate Medium	0	1	37	59	223	395	39	144	203

At the same time a tube of 1% Tryptone Water CM87, should be inoculated for the production of indole after 24 hours at 44°C.

The production of gas from lactose at 44°C and the production of indole at 44°C are accepted in the United Kingdom as evidence of *Esch. coli.*

Samples of chlorinated water giving "presumptive positive" tubes must be tested to exclude false positive results due to aerobic or anaerobic spore-bearing organisms that produce gas. Sub-cultures are made into Brilliant Green Bile (2%) Broth and incubated at 35°C for 48 hours. Production of gas within 48 hours can be taken as sufficient confirmation that coliform organisms are present. If the tubes are sub-cultured to MacConkey Agar CM7 at the same time, the colonial morphology of the organisms can easily be obtained for further differential tests.

A further multi-laboratory trial has demonstrated the efficiency of Lauryl Tryptose Mannitol Broth as a single tube confirmatory test of *Esch. coli.*[12]

Modified Direct Plate Method for Counting *Esch. coli* in Food

A direct plate method (DPM) for the rapid enumeration of *Esch. coli* in foods has been described.[13] This method was modified by adding a resuscitation procedure using Minerals Modified Glutamate Agar.[14] In the modified method 15 g of agar per litre is added to Oxoid Minerals Modified Glutamate Broth CM607 plus L124. Using this resuscitation stage the authors have recovered damaged cells from frozen, dried, heat processed and low pH foods.

Abbiss *et al.*[15] made a comparative assessment of the performance of Minerals Modified Glutamate Medium against three other enrichment broths in the enumeration of coliform organisms present in soft cheese, cooked meat and pâté. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulphate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Escherichia coli (acid + gas) ATCC® 25922

Negative control:
Enterobacter aerogenes (acid only) ATCC® 13048

Precautions

Presumptive positive tubes must be sub-cultured to Lauryl Tryptose Mannitol Broth CM831 and incubated at 44°C to detect indole formation at this temperature before the identification of *Escherichia coli* can be made.

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Lauryl Tryptose Broth
(Lauryl Sulphate Broth)
Code: CM451

A medium for the detection of coliform organisms in water and waste water, according to the formula of the American Public Health Association.

Formula

	grams/litre
Tryptose	20.0
Lactose	5.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.75
Potassium dihydrogen phosphate	2.75
Sodium lauryl sulphate	0.1

pH 6.8 ± 0.2

Directions

Dissolve 35.6 in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham). Sterilise by autoclaving at 121°C for 15 minutes.

Description

Lauryl Tryptose Broth is a selective medium which is used for the detection of coliform organisms in water, dairy products and other foods. The APHA[1] recommend that Lauryl Tryptose Broth should be used for the Mean Probable Number Presumptive Test of coliforms in waters, effluent or sewage, as a confirmatory test of lactose fermentation with gas production for milk samples (APHA)[2] and for the detection of coliforms in foods (APHA).[3]

Surface active agents have long been used as the inhibitory ingredients in selective media. MacConkey[4] introduced bile salts for this purpose and later Albus and Holm[5] working with lactobacilli found that sodium ricinoleate exerted a selective action. The development of synthetic wetting agents opened up new fields of investigation. Mallmann and Darby,[6] after comparative tests with a large number of these compounds, showed that sodium lauryl sulphate gave the best results in selective media for the coliform group.

Lauryl Tryptose Broth was designed to promote a rich growth and copious gas production from small inocula of coliform organisms. Aerobic sporing bacteria are completely inhibited. The advantage in using this product is that, in addition to giving the fermentation reaction typical of MacConkey Broth, it can also be directly tested for the presence of indole. Unlike MacConkey Broth, the medium contains no indicator, but this can be added (if required) after incubation.

Technique

For details of the APHA standard methods please consult the references.

Lauryl Tryptose Broth is recommended for the detection and enumeration of coliform organisms in water and milk products, especially in the control of ice-cream manufacture and in dairy hygiene. A suggested procedure (Dyett[7]) is as follows:

Inoculate samples of ice-cream into tubes of Lauryl Tryptose Broth in the manner normally employed in the MacConkey test. Examine the tubes after overnight incubation at 35°C and, if no gas is visible, examine again at the end of 48 hours' incubation. For every tube showing fermentation ("primary fermentation") two further tubes of Lauryl Tryptose Broth are inoculated from a tube of the primary fermenting broth, and these are incubated at 35°C and 44°C respectively. It is advisable that the tube to be incubated at 44°C is first warmed to this temperature in a water bath before inoculation.

If the 44°C incubated broth ferments after seven hours, test for indole production with either Ehrlich's or Kovac's reagent. Due to the lauryl sulphate present, shaking the reagent culture mixture forms a persistent emulsion which may interfere with the test. If fermentation has not occurred after 7 hours, leave the tube overnight at 44°C and test the following day. A positive indole reaction in a broth that has produced gas at 44°C indicates the presence of *Escherichia coli*. The tube at 35°C is incubated for 24 hours. If no fermentation occurs, the primary fermentation is assumed to be due to organisms other than coliforms. False positives are not uncommon in the primary fermentation tubes, due to fermentation of the sucrose in the added ice-cream by organisms other than coliforms.

After the two tubes of Lauryl Tryptose Broth have been inoculated for secondary fermentation, test the original primary fermentation tube (which was inoculated directly with ice-cream) for indole production. A positive reaction suggests the presence of *E. coli* and confirmation will be obtained later with the secondary fermentation from the 44°C bath. A negative indole reaction in the primary fermentation tube indicates the absence of *E. coli*.

MUG Reagent BR71 – The addition of 4-methylumbelliferyl-β-D-glucuronide (MUG) BR71 to this medium will enhance the detection of *Escherichia coli*. See MUG Reagent page 38.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at room temperature (18–22°C).

Quality Control

Positive control:
Escherichia coli ATCC® 25922 (Gas 35°C and gas and indole 44°C)

Enterobacter aerogenes ATCC® 13048 (Gas 35°C and no indole at 44°C)

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

If stored at 2–8°C the broth will become cloudy or form a precipitate. This should clear at room temperature but gas formation is the criterion of growth, not turbidity.

References

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EE Broth
Code: CM317

An enrichment medium for Enterobacteriaceae used in the bacteriological examination of foods.

Formula

	grams/litre
Peptone	10.0
Glucose	5.0
Disodium hydrogen phosphate anhyd.	6.45
Potassium dihydrogen phosphate	2.0
Ox Bile purified	20.0
Brilliant green	0.0135

pH 7.2 ± 0.2

Directions

Add 43.5 g to 1 litre of distilled water. Distribute 100 ml quantities in 250 ml flasks and heat at 100°C for 30 minutes only. Cool rapidly in cold running water. This medium is heat sensitive. ***Do not autoclave***.

Description

EE Broth (Buffered glucose–Brilliant Green bile broth) is recommended as an enrichment medium for *Enterobateriaceae* in the bacteriological examination of foods[1] and animal feed stuffs.[2] This medium is more inhibitory to non-Enterobacteriaceae than other non-selective media, e.g. Mannitol broth[3] or Lactose broth[4] by virtue of the presence of brilliant green and bile salts in the preparation.

The enumeration of Enterobacteriaceae is of great importance in monitoring the sanitary quality of food and drugs but the reliability of the methods used depends upon resuscitation of damaged cells. Such weakened cells may arise from exposure to dehydration, low pH and other unfavourable conditions.[5]

Incubation for 2 hours in well-aerated Tryptone Soya Broth CM129 (see page 44) at 25°C should precede enrichment in EE Broth. This procedure is recommended for dried foods,[6] animal feeds[7] and semi-preserved foods.[8] Occasionally, with a particular dry product, a longer incubation period is necessary, but never over 8 hours of resuscitation.

Oxoid EE Broth was formulated to overcome the unsatisfactory effects of inhibition on small numbers of Enterobacteriaceae cells due to bile salt variations. The inclusion of purified ox bile eliminated these problems and a preliminary assay can be used to check growth by inoculating approximately one viable cell per medium unit.[9,10]

For the bacteriological evaluation of processed foods the entire Enterobacteriaceae group can be used as indicator organisms.[10] This will overcome the discrepancies that can arise when lactose-negative, anaerogenic lactose-positive or late lactose-fermenting Enterobacteria are present but are missed by the standard "coli-aerogenes" tests. To overcome these problems lactose media have been replaced by those containing glucose. Mossel *et al.*[1] cited several examples in the literature which referred to various foods contaminated with salmonellae, although results for coliforms were negative. A later example quoted by Mossel[9] involved an outbreak of diarrhoea caused by French mould-fermented soft cheese contaminated by *Escherichia coli* serotype 0124. This organism is lactose-negative and therefore was not detected in coliform tests but only recognised when the commodity was tested for Enterobacteriaceae since it fermented glucose rapidly.

EE Broth should be used as an enrichment broth in conjunction with Violet Red Bile Glucose Agar CM845. When specific organisms, rather than Enterobacteriaceae in general are required subcultures must be made onto lactose differential media, e.g. Desoxycholate Citrate Agar CM35, Brilliant Green Agar CM329, or MacConkey Agar CM7 for the detection of lactose-negative or delayed organisms.

Sample size should not be less than 10 grams to yield the organisms being sought.

Technique

1 Resuscitate debilitated cells by incubating 1:10 dilutions of the food samples under investigation in Tryptone Soya Broth CM129 at 25°C for 2–8 hours. The fluid layer should not be much deeper than one centimetre. Shake the flask to disperse the contents alternately in clockwise and anti-clockwise directions for 30 seconds on three successive occasions.

2 After the period of time necessary for resuscitation, ten-fold volumes of EE Broth are added to the resuscitated suspensions.

3 Shake to disperse as above. For large samples it is desirable to add the resuscitation medium containing the product under examination, to equal volumes of double-strength EE Broth.

4 Incubate at:
44°C for 18 hours for thermotrophic bacteria
32°C for 24–48 hours for mesophilic bacteria
4°C for 10 days for psychrotrophic bacteria
depending on the groups of Enterobacteriaceae sought.

5 Examine the tubes of broth and look for turbidity with some change of colour towards yellowish-green for presumptive evidence of Enterobacteriaceae.

6 Subcultures can be made on Violet Red Bile Glucose Agar CM485 or on lactose-containing media for confirmation of lactose-fermenting or non-lactose-fermenting status. Further tests must be made to confirm the identity of the isolate.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared broth at 2–8°C.

Quality Control

Positive control:
Yersinia enterocolitica NCTC 10460
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

Avoid overheating the medium, especially the double-strength broth.

References

References are listed on the following page.

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EC Broth
Code: CM853

Formula

	grams/litre
Tryptone	20.0
Lactose	5.0
Bile Salts No. 3	1.5
Dipotassium phosphate	4.0
Mono-potassium phosphate	1.5
Sodium chloride	5.0
Novobiocin	20.0 mgm
pH 6.9 ± 0.2	

EC Broth was developed from buffered lactose broth by Perry and Hajna[1] to improve methods for detection of coliforms and *E. coli.* Tryptose was substituted for tryptone because of its superior nutritional qualities. 0.15% of bile salts No. 3 was added to inhibit Gram-positive sporulating bacilli and faecal streptococci. The bile also enhanced the growth of *E. coli.*

EC Broth is a standard methods medium.[2] With novobiocin added it is used as an enrichment medium for the detection of *E. coli* 0157.[3] (See page 45.)

References

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Tryptone Phosphate (TP) Broth
Code: CM283

Formula

	grams/litre
Tryptone	20
Dipotassium hydrogen phosphate	2
Potassium dihydrogen phosphate	2
Sodium chloride	5
Water	1000 ml
pH 7.0 ± 0.2	

Reference

Mehlman, I.J. and Romero, A. *Food Technology,* March 1992, 73–79.

Glucose-Lactose-Tween 80 (GL80) Medium

Formula

	grams/litre
Tryptone	20
Glucose	1
Lactose	4
Dipotassium hydrogen phosphate	4
Potassium dihydrogen phosphate	1.5
Sodium chloride	5
Tween 80 (Sorbitan monooleate)	1.5 ml
Water	1000 ml
pH 7.0 ± 0.2	

Reference

Mehlman, I.J. and Romero, A. *Food Technology,* March 1992, 73–79.

A brief discussion of Tryptone-phosphate (TP) broth and Glucose-lactose-Tween 80 medium (GL80) is on page 10.

Agar Media for *Escherichia coli*

Detection of *E. coli* is necessary for its role as an index and indicator organism as well as its role as a pathogen. This situation, as well as the tendency over the years for media to be devised because of deficiencies observed in earlier ones, has led to the considerable number of formulae that exist. Most of the emphasis has been placed on the role of index and indicator organisms; it is only with the recent recognition of the consequences of infection with enterohaemorrhagic strains that serious and sustained efforts have been made to provide media that are optimal for isolation of *E. coli* enteric pathogenic strains.

A study[1] to devise media better suited for isolation of the other pathogenic groupings (virotypes) resulted in the development of tryptone-phosphate-bile salts-yeast extract agar (TPBY). In this medium, based on tryptone bile agar, Tween 80 is substituted for part of the bile content in order to minimise toxicity. Additional nutrition is provided by incorporating brain-heart infusion. The medium at 44°C supported the growth of all the pathogenic strains tested. However, the medium appears to have gained limited acceptance and in general it is still necessary to use media better suited to other purposes and to carry out tests on isolates to prove their pathogenic status.

The early recognition that *E. coli* and the coliforms tolerate bile and ferment lactose forms the basis of many selective and differential media. The origins of many formulae can be traced to developments following the introduction by MacConkey[2] of the medium that carries his name. Over time, a number of variants differing in selectivity have been introduced that increase the versatility of the medium. MacConkey's medium is the basis of violet red-bile agar, the most commonly used *E. coli*/coliform detection medium.

Levine[3] took an existing medium containing eosin and methylene blue and modified it for use in water bacteriology for differentiating *E. coli* and *Enterobacter aerogenes*. Incubation of eosin-methylene blue agar (EMB) at 44°C to enhance recovery of *E. coli* was proposed in 1972.[4] Eosin methylene blue agar is used in the United States Department of Agriculture conventional culture procedure for detection of *E. coli* 0157:H7.[5]

Other early formulae are still in common use. Endo agar, which was originally devised for the isolation of *Salmonella typhi*, is frequently used to differentiate lactose-fermenting and non-lactose-fermenting intestinal organisms. Production of both acid and aldehyde by *E. coli* is responsible for the characteristic red colour of the colonies and surrounding medium.

China blue-lactose agar containing aniline blue is a non-inhibitory medium formulated by the German dairy industry in the 1960s for differentiation of lactose-fermenting and non-lactose-fermenting organisms. Gram-positive species grow well on this medium and it may be used for the detection of staphylococci and streptococci as well as differentiation of the coli-aerogenes group.

A somewhat older medium, Tergitol-7 agar, in which the surfactant Tergitol-7 is used as a selective agent is still widely used. Tri-phenyl-tetrazolium chloride (TTC) is added to allow earlier recognition and identification of *E. coli* (and *Enterobacter aerogenes*) because, exceptionally amongst coliforms they do not reduce the TTC to red formazan. The medium has been recommended for examining food materials for faecal contamination and is capable of detecting *E. coli* after 6–10 hours of incubation.[6]

The dairy and water industries traditionally have made considerable use of liquid media for detecting and enumerating *E. coli* and coliforms using the Most Probable Number (MPN) procedure. Anderson and Baird-Parker[7] recognised the disadvantages of MPN procedures when applied to examining foods and after consideration of alternative methods developed a direct count method based on that devised by Delaney, McCarthy and Grasso[8] for examining water samples. It exploits the ability of *E. coli* to produce indole when growing at 44°C on a cellulose acetate membrane placed on a bile-containing medium in a petri dish. The method was further developed to introduce a resuscitation step to allow injured cells to recover.[9] In this modification the membrane is first applied to minerals modified glutamate agar before transfer to tryptone bile agar. As with most media for *E. coli* the formula was designed to detect *E. coli* as an index and indicator organism and serological testing is required to determine whether any isolates are putative pathogens.

Hall[10] noted that the method of Anderson and Baird-Parker had some disadvantages, notably the cost of membrane filtration, and described an alternative procedure for use in confirming the presence of *E. coli* in frozen foods. The sample is inoculated on a resuscitation agar (R) containing sodium pyruvate and sodium glycerophosphate and after a period of time for recovery to occur an overlay of YLTB selective agar is added. YLTB agar contains bile salts No. 3 for selectivity and lactose and bromothymol blue to detect lactose fermentation. The selective action of the medium is enhanced by incubation at 44°C. Subsequent testing for indole production is carried out in capillary tubes on individual colonies.

Fluorogenic and chromogenic substrates which are hydrolysed by specific enzymes are increasingly used in culture media. The fluorescent and coloured hydrolysis products make possible rapid visual detection and presumptive identification of organisms that possess the enzymes. Members of the coliform group produce beta-galactosidase. *E. coli* strains, with a few exceptions, produce glucuronidase. This enzyme is not shared with coliforms and consequently detection of the enzyme can be used to differentiate *E. coli* from coliforms by observing the colour of the hydrolysis products shown by the colonies.

A number of media are available that will simultaneously detect coliforms and *E. coli*. In a comparison of Oxoid *E. coli*/coliform medium with a similar medium and a standard procedure[11] it was found that *E. coli* and coliforms could easily be counted on a single plate of the Oxoid medium. *E. coli*/coliform medium was shown to be a suitable alternative to the standard method.

The Public Health Laboratory Service (PHLS) has produced a medium based on tryptone-bile agar. This medium, known as TBX, contains the glucuronidase substrate 5-bromo-4-chloro-3-indoxyl β-D-glucuronic acid (BCIG). Hydrolysis of this compound by *E. coli* produces blue colonies. An unpublished report by the PHLS of a trial of the Oxoid medium showed there to be a high level of agreement between the results given by TBX and tryptone-bile agar. Use of TBX medium shows distinct advantages over the British Standard method including confirmation of *E. coli* without the need for additional reagents.

Chromogenic agars specifically for *E. coli* 0157 have now been introduced.

Rappaport and Henig[12] remarked in a paper written in 1952 that the connection between *E. coli* and enteric disease had first been observed in 1923 but at the time of writing still the only means of recognising pathogenic strains was to use antisera to place isolates into serological groupings known to be associated with disease. Their particular interest was in serotypes 055 and 0111 and, following an earlier observation that these two serotypes fermented sorbitol slowly or not at all, they substituted sorbitol for lactose in MacConkey agar in order to detect strains of 055 and 0111 by their growth as colourless colonies because of their failure to ferment sorbitol. Little further work was done on formulating agar media

specifically for enteropathogenic *E. coli* strains until the stimulus provided by the recognition of serotype 0157:H7 as the cause of a new disease syndrome, haemorrhagic colitis. Marsh and Ratnam[13] observed that serotype 0157 also was unable to ferment sorbitol and investigated the usefulness of a suggestion[14] that the sorbitol MacConkey agar described by Rappaport and Henig could be used in the isolation of *E. coli* 0157 from diseased patients. The medium was found to be reliable and has now become the preferred medium for this purpose. Continuing experience showed that sorbitol MacConkey agar is frequently insufficiently selective and improvements in selectivity have been reported, first by the incorporation of cefixime and rhamnose[15] and subsequently by the addition of potassium tellurite at a concentration that inhibits growth of *E. coli* with the exception of serotype 0157.[16] The much improved selectivity has diminished the importance of rhamnose as a differential agent and it is now usual to omit it from the medium because of the high cost of this carbohydrate.

Despite the adjustments made in selectivity to optimise detection methods using sorbitol MacConkey agar;[17,18] demonstration of a range of susceptibilities to cefixime and potassium tellurite by different strains of *E. coli* 0157[19] may explain the currently poor isolation rate from foods, particularly if the cells are stressed.

Work by Czechowicz[20] and colleagues has confirmed the value of pyruvate in recovery of heat-stressed cells of *E. coli* 0157 on tryptone soya agar supplemented with 1% sodium pyruvate. Recovery of the cells using the spread-plate method was greater than using the pour plate method. Future work directed at improving performance of cefixime-tellurite-sorbitol MacConkey agar might show advantages in using tryptone soya-pyruvate agar in a solid medium repair procedure before overlaying with the selective medium.

Other refinements have been made to the methodology using sorbitol MacConkey agar. *E. coli* 0157 is unusual amongst *E. coli* in not producing glucuronidase and this characteristic has been exploited by the incorporation in the medium of the fluorogenic substrate 4-methylumbelliferyl β-D-glucuronide[21] (MUG). Unlike strains that produce glucuronidase, *E. coli* 0157 colonies will not fluoresce when illuminated with ultra-violet light. McCleery and Rowe[22] reported that poor performance of the fluorogenic medium with stressed cells could be improved by the addition of catalase to tryptone soya agar on which samples are initially inoculated for a period of resuscitation. An overlay of the selective medium is then added. The efficacy of MUG is influenced by a number of factors including the culture medium, substrate concentration, incubation temperature and incubation time. A study designed to establish the conditions necessary for optimum use of MUG in food microbiology has recently been reported.[23]

Use of an alternative chromogen, 5-bromo-4 chloro-3-indoxyl-β-D-glucuronide (BCIG) has been reported.[24] Colonies of *E. coli* 0157 that are present on sorbitol MacConkey agar containing this chromogen appear white, while sorbitol-negative, β-glucuronidase-positive colonies are coloured green to blue.

Immunomagnetic separation (IMS) to concentrate cells in enrichment culture prior to plating on cefixime-tellurite-sorbitol MacConkey agar is now an established procedure.[17,18,25] Incubation of the enrichment broth at 44°C further enhances selectivity although for some strains this temperature may be too high. Immunomagnetically separated cells may also be detected by immunoassay.[25]

Despite the obvious usefulness of immunomagnetic separation for the detection of *E. coli* 0157:H7, continuing reports of non-0157 serogroups as a cause of haemolytic-uraemic syndrome point to the need to employ additional methods besides IMS in the bacteriological diagnosis of this condition and attempts to isolate the organism causing it.[26]

A method for isolating verocytotoxin-producing *E. coli* of any serotype directly from samples and mixed cultures has been reported.[27] Samples and toxin-positive mixed cultures are inoculated onto hydrophobic grid membrane filters placed on the agar medium. A second membrane placed beneath the hydrophobic membrane localises toxin secreted from verocytotoxigenic strains beneath the colonies by immunocapture. Removal and immunostaining of the second membrane reveals dots of captured toxin corresponding to the position of colonies on the hydrophobic membrane. These colonies can be picked directly for further investigation. The authors claim that this technique enables isolation of any verocytotoxigenic serotype from food and faecal samples in 24 to 48 hours.

Sorbitol MacConkey agar is used in a membrane lifting technique[28] in which nitrocellulose membranes that have been pressed into contact with ground meat samples are incubated on the medium. Immunostaining of the membranes with horse radish peroxidase-conjugated goat-anti-*E. coli* 0157:H7 antibodies after overnight incubation at 37°C enabled colonies of *E. coli* 0157:H7 to be identified rapidly amongst the other flora present.

Tryptone bile agar was taken by Szabo, Todd and Jean[29] as the basis of a new medium (HC medium) for direct overnight isolation of haemorrhagic colitis (HC) strains of *E. coli* from foods. Tryptone bile agar was chosen for its reliability in detecting indole production. Modifications to the medium were made to exploit the inability of haemorrhagic colitis strains to produce glucuronidase and their failure to ferment sorbitol; characteristics which distinguish HC strains from other *E. coli*. The bile content is reduced and sodium chloride is added for its protective effect during incubation at 45.5°C. Bromocresol purple is present as a pH indicator. The agar is used with filter membranes similarly to tryptone bile agar. Lack of fermentation of sorbitol causes HC strains to appear as blue colonies in contrast to the yellow colonies of other strains. HC strains give red colonies when tested for indole, very different from the yellow appearance of other strains. This apparent failure to produce indole occurs because sorbitol-fermenting strains utilise sorbitol preferentially to tryptophan. On prolonged incubation colonies gradually become positive as the sorbitol content of the medium is depleted and eventually exhausted. Colonies of HC strains do not fluoresce under ultra-violet light because of their failure to hydrolyse MUG.

HC agar is specified in Method B of the FDA-BAM procedures for isolation of enterohaemorrhagic *E. coli*. (See page 42.)

Entis and Lerner[30] have found the methodology using HC agar to be excessively labour-intensive and have developed SD-39 agar which incorporates sorbitol fermentation, lysine decarboxylation and glucuronidase production as differential biochemical reactions which can be read simultaneously, instead of sequentially as is necessary with HC agar.

Selectivity is provided by novobiocin and monensin with incubation at 44°C to 44.5°C. Sodium chloride present protects *E. coli* 0157 from the inhibitory effect of this temperature.

Detailed descriptions of Oxoid agar media for coliforms and *E. coli* are given on pages 19–53 and sorbitol-MacConkey agar for *E. coli* 0157 on page 46.

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Violet Red Bile Lactose Agar

Violet Red Bile Lactose Agar
Code: CM107

A lactose-containing selective medium for the detection and enumeration of coliform organisms in water, food and dairy products.

Formula

	grams/litre
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile salts No. 3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 ± 0.2	

Directions

Suspend 38.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. No further sterilisation is necessary or desirable. Mix well before pouring.

Description

Violet Red Bile Lactose Agar is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the *coli-aerogenes* content of water, milk and other dairy products, dairy equipment, and food products etc.[1,2]

Organisms which rapidly attack lactose produce purple colonies surrounded by purple haloes. Non-lactose or late-lactose fermenters produce pale colonies with greenish zones. Other related Gram-negative bacteria may grow but can be suppressed by incubation at >42°C or by anaerobic incubation.

Druce *et al.*[3] found that Violet Red Bile Lactose Agar was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey Broth, and that the Oxoid medium was suitable for determining the *coli-aerogenes* content of milk.

Technique

Druce *et al.* recommended the following procedures:

For the routine determination of the *coli-aerogenes* content of raw milk, prepare pour-plates containing 1.0, 0.1 and 0.01 ml of the sample in Violet Red Bile Lactose Agar, and incubate for 20–24 hours at 35°C.

For *coli-aerogenes* counts of pasteurised milk, employ four pour-plates of Violet Red Bile Lactose Agar. Divide 10 ml of the sample among three of the plates, and add 1 ml of the sample to the remaining plate. Incubate for 20 to 24 hours at 30°C. Similarly the examination of rinses and swabs from dairy equipment and apparatus, should include the spreading of 10 ml of solution on each of three plates and of 1 ml on a single plate. Coliform organisms form dark red colonies which are 1 to 2 mm in diameter, usually surrounded by a reddish zone. Occasionally *coli-aerogenes* may be considerably smaller (less than 0.5 mm in diameter).

When preparing pour-plates the medium should be freshly made up, cooled to 47°C and used within 3 hours.

An overlay method is helpful to improve the specificity of the medium. In this case a thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation may be carried out at >42°C for 18 hours, 32°C for 24–48 hours or 4°C for 10 days, depending on the temperature characteristics of the organisms to be recovered. For *E. coli* a temperature of 44° ± 1°C is specifically recommended.[4]

Characteristic Appearance of Colonies

Round, purple-red <0.5–2 mm may be surrounded by purple-red haloes (lactose-positive organisms) pale, may have greenish haloes (lactose-negative organisms).

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C and use as freshly as possible.

Quality Control

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

This medium is not completely specific for Enterobacteriaceae, other organisms e.g. *Aeromonas* and *Yersinia* species may give similar reactions. The selectivity of the medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

References

1 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th Edn. APHA Inc. Washington D.C.
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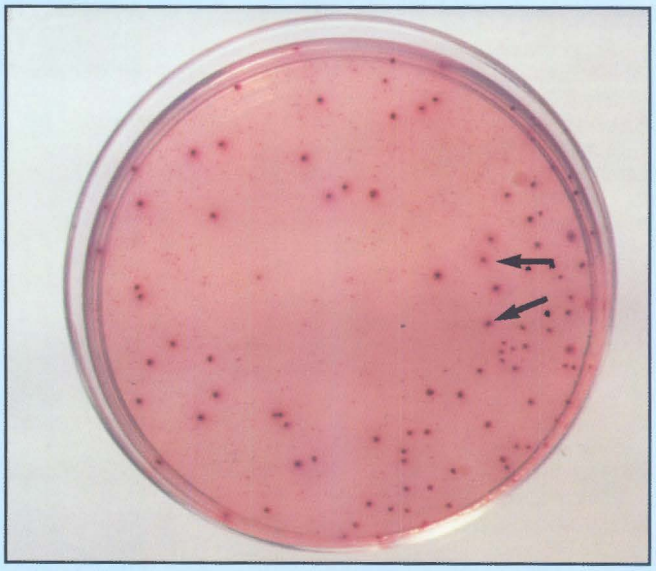

Appearance of *E. coli*, (arrowed) on Violet Red-Bile-Lactose agar. Pour plate. Note the large number of small non-lactose-fermenting colonies.
Oxoid Marketing Collection.

Violet Red Bile Glucose Agar

Violet Red Bile Glucose Agar
Code: 485

A glucose-containing selective medium for the detection and enumeration of Enterobacteriaceae in food products.

Formula

	grams/litre
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile salts No. 3	1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 ± 0.2	

Directions

Suspend 38.5 g in 1 litre of distilled water. Boil to dissolve the medium completely. No further sterilisation is necessary or desirable. Mix well and dispense into tubes or dishes.

Description

Results from tests that may be applied to water to detect *coli-aerogenes* organisms as possible indicators of faecal contamination possess far less significance when applied to raw foods. In the examination of foodstuffs, detection of a more defined group of organisms, the Enterobacteriaceae, that ferment glucose to produce acid and/or gas has been recommended.[1,2] In addition to coliforms this group includes salmonellae and shigellae, which do not ferment lactose, and enterotoxigenic *E. coli*. It also contains organisms such as *Klebsiella* and *Citrobacter*, which are more resistant to heat than coliforms and are therefore better indicators of failure of processes that use minimal heat.

The difficulties of measuring the total Enterobacteriaceae content of foodstuffs have been studied by Mossel *et al.*,[3] who showed that the addition of glucose to an existing medium for the detection of coliforms improves the performance. They added 10 g per litre of glucose to crystal violet neutral red bile lactose agar (Violet Red Bile Agar CM107), and named the modified formulation MacConkey Glucose Agar.

Further work by Mossel *et al.*[4,5] showed that the lactose could be omitted resulting in the formulation of Violet Red Bile Glucose Agar CM485. The continued inclusion of lactose would not provide test results leading to more accurate identification. Exclusion of lactose renders the medium more economical to make as less weight is required per litre.

Media that contain bile salts have an intrinsic toxicity for Enterobacteriaceae, even for cells that have not been under stress.[6,7,8,9,10,11]

Considerable differences have been observed among six commercial preparations of Violet Red Bile Agar with regard to productivity for Enterobacteriaceae,[12] and the intensity of their metabolism. In conjunction with Oxoid the components of the medium were examined and Mossel drafted a specification as follows:

1 Approved media have to be clear and yield colonies of satisfactory size. They have to give reproducible counts of typical colonies of Enterobacteriaceae.

2 When challenged for intrinsic toxicity by an anaerobic metabolic test using a strain of *Yersinia enterocolitica* (Serotype 03) as a sensitive indicator, media must promote adequate growth, acid formation and, where required, adequate gas formation.[13]

3 Media have to satisfy the confirmation rate of typical colonies, i.e. the number of colonies confirmed as Enterobacteriaceae divided by the number of colonies tested.

Violet Red Bile Glucose Agar CM485 has been developed to satisfy all of these criteria and complies with the recommendations of ISO.[14]

Technique

Prepare a series of dilutions of the samples so that at least one will be included that will yield 100–200 colonies from a 1 ml aliquot. Transfer 1 m aliquots of each dilution to 9 cm petri dishes using two plates for each dilution. Add 15 ml of medium cooled to 47°C. Gently swirl the plates three times clockwise and three times anti-clockwise. After the medium has solidified overlay with 10 ml of the same medium and leave to solidify. Invert the dishes and incubate at >42°C for 18 hours, 32°C for 24–48 hours or 4°C for 10 days depending on the groups of Enterobacteriaceae to be recovered.

Violet Red Bile Glucose Agar is heat sensitive and must not be held molten for more than 3–4 hours before use.[15]

The agar overlay ensures anaerobic conditions which suppress the growth of non-fermentative Gram-negative bacteria. It also encourages the fermentation of glucose which favours the formation of clearly visible purple colonies, surrounded by a purple halo.

Characteristic Appearance of Colonies

Round, purple 1–2 mm diameter surrounded by purple haloes. Colonies may sometimes be 0.5 mm or less. Confirmation of the identity of colonies must be made by further tests.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C and use as freshly as possible.

Quality Control

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

This medium is not completely specific for Enterobacteriaceae, other organisms may grow, e.g. *Aeromonas* and *Yersinia species*.

The selective activity of this medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

Medium for the poured plate procedure should be freshly prepared, cooled to 47°C and used within 3 hours.

References

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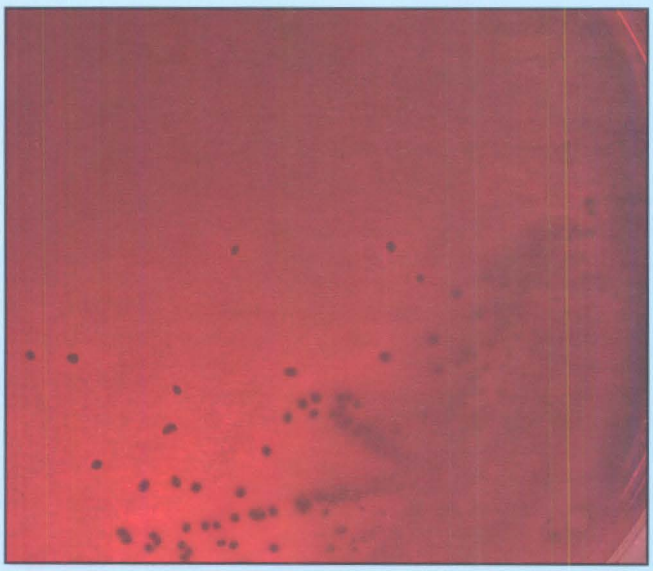

Appearance of *E. coli* on Violet Red-Bile-Glucose agar. The growth is covered with an overlay of culture medium. All members of the Enterobacteriaceae grow as red colonies on this medium.

Violet Red Bile Agars

A Guide to the Choice of an Appropriate Medium

Media containing bile and the dye violet red used in the examination of foods are based on the medium developed by MacConkey for detection of bile-tolerant Gram-negative bacteria. Violet Red Bile Agar CM107 (VRBA) contains lactose as a fermentable carbohydrate which enables the medium to be used for the detection of lactose-fermenting bacteria and differentiation of the group of bacterial genera known as coliforms or coli-aerogenes bacteria from non-lactose-fermenting organisms. Violet Red Bile Glucose Agar CM485 (VRBGA) differs from VRBA only by substitution of glucose for lactose. By definition all members of the Enterobacteriaceae ferment glucose and grow as purple-red "positive" colonies.

VRBA was used routinely during the years 1925 to 1935 to monitor the efficacy of milk pasteurisation and milking parlour hygiene. It is still considered to be a useful medium for testing the hygienic status of water and milk. All Gram-negative bacteria capable of growth on bile-containing media and which ferment lactose are included in the coliform count. Lactose-fermenting colonies must not be assumed to be *E. coli* and if required colony identification should be carried out.

Extension of the use of VRBA into food examination revealed weaknesses in using the ill-defined "coli-aerogenes" group of organisms for assessments of processing and general hygiene. Lack of standardisation of methodology and differences in interpretation of colony morphology of coli-aerogenes organisms sometimes led to very considerable differences in accuracy and precision of results. This situation is brought about because generally all Gram-negative bacteria capable of growth on bile-containing media and which ferment lactose are included in the coliform count. This mixture of organisms may vary because of a number of influences including the type of sample under investigation, the culture medium used, incubation temperature and criteria chosen for reading the results. This last issue might erroneously exclude relevant organisms because of an unusual colony appearance; such as colour, size and presence or absence of bile precipitation surrounding colonies.

Substitution of glucose for lactose in the same selective medium resulted in Violet Red Bile Glucose Agar CM485. All Enterobacteriaceae on this medium produce "purple-red" colonies and use of the clearly delineated family of Enterobacteriaceae eliminates the inaccuracies in methods and interpretation inherent in the ill-defined "coli-aerogenes" group. However, there is the possibility of falsely reassuring results in situations where lactose-negative organisms predominate and where these may include non-lactose-fermenting pathogens, e.g. *Salmonella* species. In summary, generally little is to be gained by continued use of "coli-aerogenes" bacteria instead of Enterobacteriaceae as index or indicator organisms but much may be lost. Violet Red Bile Glucose Agar is becoming the preferred medium for use in many investigations into raw materials, processed foods and plant hygiene. However, when testing for example, raw vegetables, Violet Red Bile Agar may remain a more practical choice for the assessment of their hygienic status because certain non-lactose-fermenting but glucose utilising organisms, e.g. *Pseudomonas* species predominate amongst the naturally-occurring associated flora and may easily overgrow the indicator organisms on VRBGA.

If there is any concern that non-lactose-fermenting pathogens may also be present, then consideration should be given to performing additional specific tests for these organisms, if necessary by a consulting laboratory.

Further information about the significance of Enterobacteriaceae present may be obtained by incubating VRBGA at different temperatures. When testing for enteric pathogens is not feasible, incubation at elevated temperatures, e.g. 42–44°C for detecting populations of thermotrophic Enterobacteriaceae may be helpful because these organisms and major enteric pathogens thrive at similar temperatures. **A test for thermotrophic Enterobacteriaceae should not be regarded as a substitute for a search for specific enteric pathogens.**

Some non-Enterobacteriaceae such as *Aeromonas* species may also grow on VRBA and VRBGA. It is important to have a general understanding of the microflora likely to be encountered in any specific sample. This will assist in the selection of the most appropriate medium and also guide the microbiologist in deciding the necessity for colony identification.

Because of the multiplicity of genera designated "coli-aerogenes" and Enterobacteriaceae that grow on VRBA and VRBGA, variety may be expected in their colony appearance and size. These differences may be further influenced by parameters such as the numbers of colony forming units present, their distance from each other and by incubation temperature. Although generally colonies may be up to 2 mm in diameter, they may be considerably smaller, sometimes less than 0.5 mm. In practice, all purple-red colonies on Violet Red Bile Agar CM107 should be regarded as presumptive coli-aerogenes and all purple-red colonies on Violet Red Bile Glucose Agar CM485 as Enterobacteriaceae. If required, further tests may be carried out to confirm their identity.

Mossel[1] has carried out much of the work on the role of Violet Red Bile Agar and Violet Red Bile Glucose Agar in food microbiology and his paper is a useful introduction to the subject. Further detailed discussion and methodology for examining foods for the presence of Enterobacteriaceae and coli-aerogenes (coliform) bacteria is given by Mossel *et al.*[2]

Neither VRBA or VRBGA are intended to be used for detection of enteropathogenic *E. coli*. If it is necessary to determine whether any of the *E. coli* colonies present are of pathogenic strains then it will be necessary first to establish the serogroup and subsequently submit the isolate to tests of pathogenicity.

References

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China Blue Lactose Agar

China Blue Lactose Agar
Code: CM209

A standard, non-inhibitory solid medium for enumeration and differentiation of bacteria in dairy products.

Formula

	grams/litre
Peptone	5.0
'Lab-Lemco' powder	3.0
Lactose	10.0
Sodium chloride	5.0
China blue	q.s.
Agar	12.0
pH 7.0 ± 0.2	

Directions

Suspend 35 g in 1 litre of distilled water. Boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

China Blue Lactose Agar was formulated by Brandl and Sobeck-Skal.[1] It is a standard, non-inhibitory solid medium for the differentiation and enumeration of bacteria in milk, proposed by the Methodenkommission für Milchwirtschaft.[2] The china blue serves as a pH indicator to differentiate between lactose fermenters and non-lactose fermenters but does not suppress the growth of cocci; therefore this medium may be used for the detection of streptococci and staphylococci as well as the coli-aerogenes group.

Plates may be streak-inoculated or decimal dilutions of milk may be added to the molten, cooled medium in a pour-plate technique.

After 18 hours incubation at 35°C colony appearances are:

Colour	***Microorganisms***
Blue	Lactose fermenters e.g. *Escherichia coli* and coliform bacteria: 3–4 mm diameter Staphylococci: 1 mm diameter Streptococci: 0.5 mm diameter.
Colourless	Non-lactose fermenters e.g. *Salmonella, Serratia, Proteus* species and others.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Enterococcus faecalis ATCC® 29212
Escherichia coli ATCC® 25922

Negative control:
Uninoculated medium

Precautions

It is important to remember that Gram-positive and Gram-negative cocci and bacilli can grow on this medium. Always confirm the organism morphology and Gram reaction.

References

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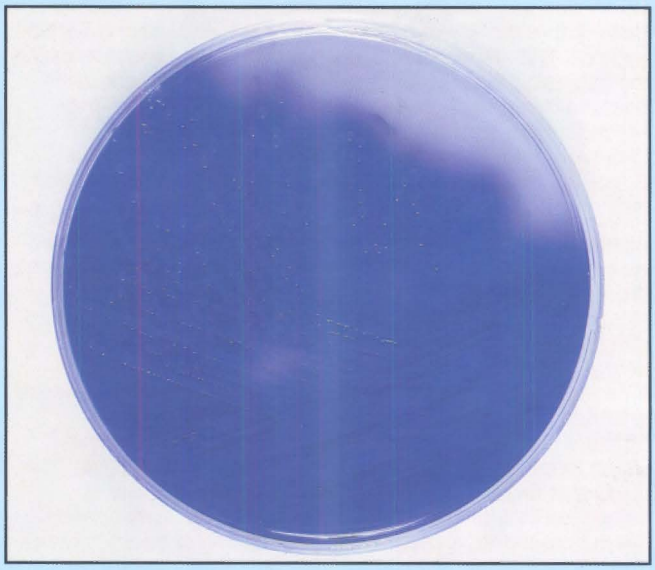

Appearance of *E. coli* on China Blue-lactose agar.
Oxoid Marketing Collection.

Desoxycholate Agar

Desoxycholate Agar
Code: CM163

A differential medium for the enumeration of coliforms in dairy products. It may be employed as a non-selective medium for the isolation of enteric pathogens.

Formula

	grams/litre
Peptone	10.0
Lactose	10.0
Sodium desoxycholate	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.0
Ferric citrate	1.0
Sodium citrate	1.0
Neutral red	0.03
Agar	15.0
pH 7.1 ± 0.2	

Directions

Suspend 45 g in 1 litre of distilled water. Bring to the boil over gauze and flame to dissolve the medium completely. Agitate to prevent charring.

This medium is heat sensitive. Avoid excessive or prolonged heating during reconstitution. Do not autoclave or remelt.

Description

Desoxycholate Agar is a differential medium for the direct count of coliforms in dairy products (American Public Health Association[1]). It may also be employed for the isolation of enteric pathogens from rectal swabs, faeces, or other specimens.

The medium may be used in a "pour-plate" technique or as a surface inoculated medium. A thin layer of uninoculated Desoxycholate Agar poured over the surface of a gelled "pour-plate" assists subsequent counting.

Technique

Enumeration of Coliforms in Milk and Cream (APHA)[1]

1 Pipette 1–4 ml of the sample (or decimal dilution of the sample) into a sterile petri dish.

2 Cool freshly prepared Desoxycholate Agar to 42–44°C and add 10–20 ml to each dish.

3 Mix the contents of the dishes by gentle tilting and rotation.

4 Allow the plates to solidify and pour on an overlay of 3–4 ml of uninoculated Desoxycholate Agar.

5 When the overlay has set, invert the plates and incubate them for 18–24 hours at 35°C.

6 Count all dark red colonies measuring at least 0.5 mm in diameter, and calculate the number of coliform colonies per millilitre or gram of original sample.

Isolation of Enterobacteriaceae

It is advisable to use Desoxycholate Agar in parallel with other plating media for this purpose.

Lightly inoculate a Desoxycholate Agar plate with faeces, rectal swab, or enrichment culture. Incubate for 18–24 hours at 35°C and examine. Non-lactose fermenters of enteric origin form colourless colonies. Non-lactose fermenters which are not of enteric origin are generally inhibited by the sodium desoxycholate in the medium. Identify suspect colonies in the usual manner.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control

Positive control:
Lactose fermenters
Escherichia coli ATCC® 25922
Klebsiella oxytoca NCTC 8167
Non-lactose fermenters
Shigella sonnei ATCC® 25931

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

As with all desoxycholate media, this medium is heat sensitive. Observe the precautions stated under Directions.

Reference

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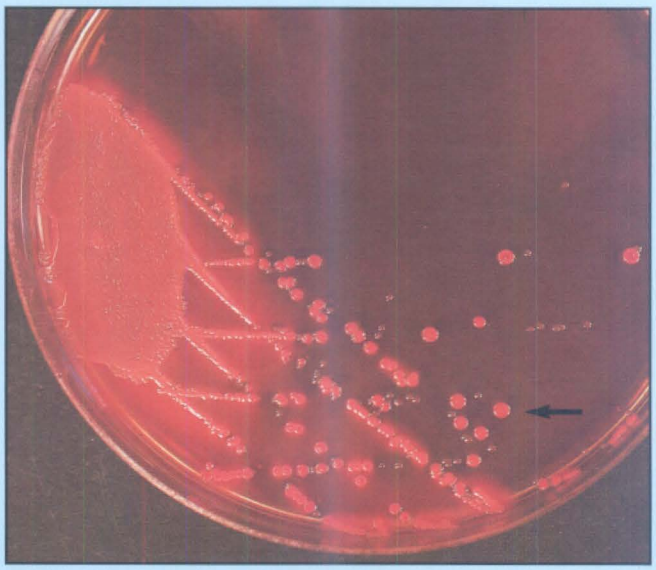

Appearance of *E. coli* (arrowed) and *Shigella spp.* on Desoxycholate Agar. *Oxoid Marketing Collection.*

Eosin Methylene Blue Agar (Modified) Levine

Eosin Methylene Blue Agar (Modified) Levine
Code: CM69

An isolation medium for the differentiation of the Enterobacteriaceae.

Formula

	grams/litre
Peptone	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.065
Agar	15.0
pH 6.8 ± 0.2	

Directions

Suspend 37.5 g in 1 litre distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

Description

This versatile medium, modified by Levine,[1,2] is used for the differentiation of *Escherichia coli* and *Enterobacter aerogenes*, for the rapid identification of *Candida albicans*, and for the identification of coagulase-positive staphylococci.

The medium is prepared to the formula specified by the APHA[3,4,5,6] for the detection and differentiation of the coliform group of organisms.

Weld[6,7] proposed the use of Levine eosin-methylene blue agar, with added chlortetracycline hydrochloride for the rapid identification of *Candida albicans* in clinical materials. A positive identification of *Candida albicans* could be made after 24 to 48 hours incubation at 37°C in 10% carbon dioxide from faeces, oral and vaginal secretions, and nail or skin scrapings. Vogel and Moses[8] confirmed the reliability of Weld's method for the relatively rapid identification of *C. albicans* in sputum. They found that use of eosin methylene blue agar was just as reliable as more conventional methods for the identification of this organism in sputum. In addition, the medium provided a means for the identification of several Gram-negative genera. Doupagne[9] also investigated the use of the Levine medium under tropical conditions.

Haley and Stonerod[10] found that Weld's method was variable so that Walker and Huppert[11] advocated the use of corn meal agar and a rapid fermentation test in addition to the Levine medium. Using the combined rapid technique they were able to obtain results within 48 to 72 hours.

Subsequent to the findings of Vogel and Moses,[8] Menolasino *et al.*[12] used Levine eosin methylene blue agar for the identification of coagulase-positive staphylococci which grew as characteristic colourless, pin-point colonies. The Levine medium was more efficient than tellurite glycine agar and showed good correlation with the plasma coagulase test.

Colonial Characteristics

Escherichia coli – isolated colonies, 2–3 mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

Enterobacter aerogenes – 4–6 mm diameter, raised and mucoid colonies, tending to become confluent, metallic sheen usually absent, grey-brown centres by transmitted light.

Non-lactose-fermenting intestinal pathogens – translucent and colourless.

Candida albicans – after 24 to 48 hours at 35°C in 10% carbon dioxide "spidery" or "feathery" colonies. Other *Candida* species produce smooth yeast-like colonies. Since a typical appearance is variable it is advisable to use a combined method such as that of Walker and Huppert.[14]

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control

Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium

Precautions

Further tests are required to confirm the presumptive identity of organisms isolated on this medium. Some strains of *Salmonella* and *Shigella* species will not grow in the presence of eosin and methylene blue. Store the medium away from light to prevent photo-oxidation.

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Endo Agar

Endo Agar
Code: CM479

A modified medium requiring the addition of basic fuchsin to form Endo Agar.

Formula

	grams/litre
Peptone	10.0
Lactose	10.0
Dipotassium phosphate	3.5
Sodium sulphite	2.5
Agar	10.0

pH 7.5 ± 0.2

Directions

Suspend 36 g in 1 litre of distilled water. Add 4 ml (or as directed by the supplier) of a 10% w/v alcoholic solution of basic fuchsin BR50 (95% ethyl alcohol). Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Basic fuchsin is a potential carcinogen and care should be taken to avoid inhalation of the powdered dye and contamination of the skin.

Plates should be stored in the dark to preserve their pale pink colour.

Description

Endo Agar is a long established medium which was originally devised for the isolation of the typhoid bacillus. More reliable media for this purpose have since been evolved, and the medium is now used for the differentiation of lactose-fermenting and non-lactose-fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms. Production of both acid and aldehyde by lactose-fermenting organisms, such as *Escherichia coli*, gives rise to the characteristic red colouration of the colony and the surrounding medium.

Technique

For the confirmation of presumptive tests with liquid media, subculture tubes showing gas, or acid and gas formation, onto an Endo Agar plate. Incubate for 24 hours at 35°C.

Lactose-fermenting coliforms (e.g. *Escherichia coli)* give rise to deep red colonies which colour the surrounding medium and possess a golden metallic sheen.

Non-lactose fermenters form colourless translucent colonies, against the pink to colourless medium.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C away from light.

Quality Control

Positive control:
Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048
Proteus vulgaris ATCC® 13315

Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

Weigh out the basic fuchsin (BR50) in a fume cupboard and avoid inhalation of the powder or contamination of the skin.

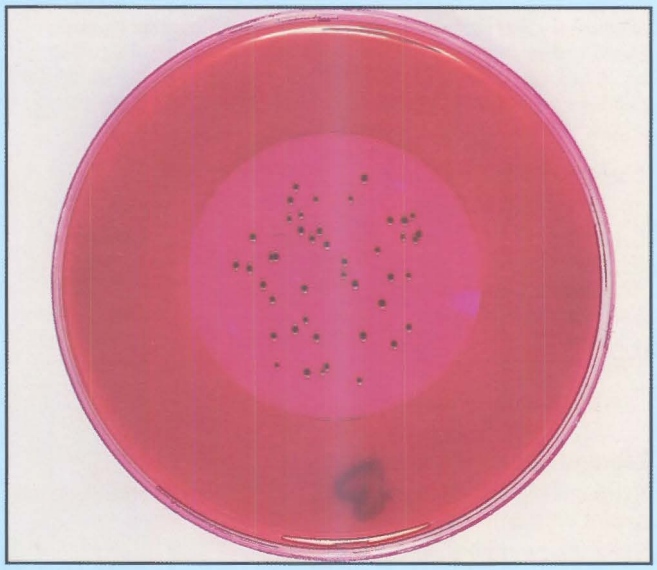
Keep the prepared medium away from light to avoid photo-oxidation.

Endo Agar is quoted by the American Public Health Association as a "Standard Methods" medium for use in water[1] and dairy products.[2] Windle-Taylor[3] recommended the medium for the isolation and differentiation of coli-aerogenes bacteria from water.

References

1 American Public Health Association (1992) *Standard Methods for the Examination of Water and Wastewater.* 18th edn. APHA Inc. Washington DC.
2 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
3 Windle-Taylor, E. (1958) *The Examination of Waters and Water Supplies.* 7th edn. Churchill Ltd., London, pp. 417, 440–441, 780–781.

Appearance of *E. coli* (arrowed) and *Shigella spp.* on Eosin-Methylene Blue Agar. Eosin-Methylene Blue Agar is described on page 28.



Appearance of *E. coli* on a filter membrane placed on Endo Agar.
Oxoid Marketing Collection.

MacConkey Agar

MacConkey Agar
Code: CM7

A differential medium for the isolation of coliforms and intestinal pathogens in water, dairy products and pathological specimens.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

pH 7.4 ± 0.2

Directions

Suspend 52 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Dry the surface of the agar before inoculation.

Description

A differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and pathological specimens. MacConkey Agar CM7 corresponds to the medium recommended by the World Health Organisation,[1] the Department of Health[2] and by Windle-Taylor[3] for the bacteriological examination of water.

Although principally used for coliforms, this medium may also be employed for the differentiation of other enteric bacteria (including pathogens) and is suitable for the differentiation of *Pasteurella species.*

Technique

Pathological Specimens

Due to its ability to support the growth of pathogenic Gram-positive cocci (e.g. Staphylococci and enterococci) as well as Enterobacteriaceae, MacConkey Agar CM7 is particularly recommended for the cultivation of pathogens which may be present in a variety of specimens such as urine, faeces and wound swabs. Whilst it is selective it does not suppress a mixed bacterial flora to the same extent as other inhibitory media (including other MacConkey agars). It provides a number of other diagnostic indications in addition to bile tolerance, such as colony morphology and chromogenesis. MacConkey Agar should be used in conjunction with other more selective media e.g. XLD Agar, when used for enteric pathogens and with blood agar when examining urines and wound swabs.

Colonial Characteristics

After 24 hours at 35–37°C typical colonies are as follows:

Organism	Colour	Remarks
Escherichia coli	Red	non-mucoid
Aerobacter aerogenes	Pink	mucoid
Enterococcus species	Red	minute, round
Shigella spp.	Colourless	non-mucoid
Staphylococci	Pale pink	opaque
Pseud. aeruginosa	Green-Brown	fluorescent growth

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Enterococcus faecalis ATCC® 29212
Escherichia coli ATCC® 25922
Shigella sonnei ATCC® 25931
Staphylococcus aureus ATCC® 25923

Negative control:
Uninoculated medium

Precautions

The colonial characteristics described only give presumptive identification of the isolated organism. It is necessary to subculture and carry out confirmation tests for final identification. To enhance the pigment of suspected *Staphylococcus aureus*, hold the plates on the bench at ambient temperature for 12–18 hours.

References

1 World Health Organisation (1963) International Standards for Drinking Water 2n. WHO, Geneva.
2 Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) The Bacteriological Examination of Drinking Water Supplies. *Report No. 71.* HMSO, London.
3 Windle-Taylor, E. (1958) *The Examination of Waters and Water Supplies* 7th edn. Churchill Ltd. London.

MacConkey Agar (without salt)
Code: CM7b

A differential medium on which swarming of Proteus species is suppressed. Recommended for urine examination and may be used for the same purposes as MacConkey Agar CM7 in the examination of food samples.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts	5.0
Neutral red	0.075
Agar	12.0
pH 7.4 ± 0.2	

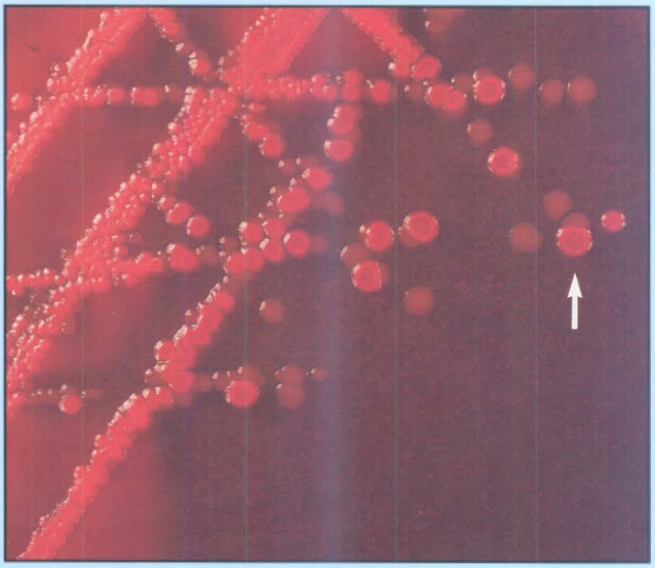
Directions

Suspend 47 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Mix well before pouring. Dry the surface of the agar before inoculation.

Description

This medium has the same formulation as MacConkey Agar CM7 except that it does not contain added salt and therefore provides a "low electrolyte medium" on which most *Proteus* species do not spread. For this reason the medium has found particular favour for use in the examination of urine so that overgrowth of other organisms is prevented.

The medium behaves similarly to MacConkey Agar CM7 when used for detection of enteric organisms.



Appearance of *E. coli* (arrowed) and *Shigella spp.* on MacConkey Agar. The appearance on MacConkey Agar without salt is similar.

MacConkey Agar No. 3

MacConkey Agar No. 3
Code: CM115

A selective medium giving excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram-positive cocci.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

pH 7.1 ± 0.2

Directions

Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

A more selective modification of MacConkey medium which is suitable for the detection and enumeration of coliform organisms and also for the detection and isolation of *Salmonella* and *Shigella species* occurring in pathological and food specimens. Due to the inclusion of a specially prepared fraction of bile salts in addition to crystal violet, the medium gives improved differentiation between coliforms and non-lactose-fermenting organisms whilst Gram-positive cocci are completely inhibited.

This formulation corresponds with that recommended by the American Public Health Association[1] for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms[2] and for the isolation of *Salmonella* and *Shigella species* in cheese.[3]

The addition of 100 mg of 4-methylumbelliferyl-β-D-glucuronide to one litre of MacConkey Agar detects the enzyme β-glucuronidase.[4] The cleaved 4-methylumbelliferyl moiety is fluorescent at 366 nm. Thus colonies of *Esch. coli* can be detected rapidly in mixed cultures by examining the plate under a uv lamp after overnight incubation at 35°C.[5] However, it should be remembered that other organisms may also be β-glucuronidase positive.

MacConkey Agar No. 3 was chosen by Rappaport and Henig[6] for the development of Sorbitol MacConkey Agar. (See page 46.)

Technique

After inoculation the plates are usually incubated for 18 to 24 hours at 35°C and for a further 24 hours if non-lactose-fermenting organisms are sought and have not appeared. Lower incubation temperatures may sometimes be used for psychrophilic species. After 18 hours at 35°C, coliforms produce intense violet-red colonies whilst non-lactose fermenters are colourless.

Storage

The dehydrated medium should be stored below 25°C and used before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Escherichia coli ATCC® 25922
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

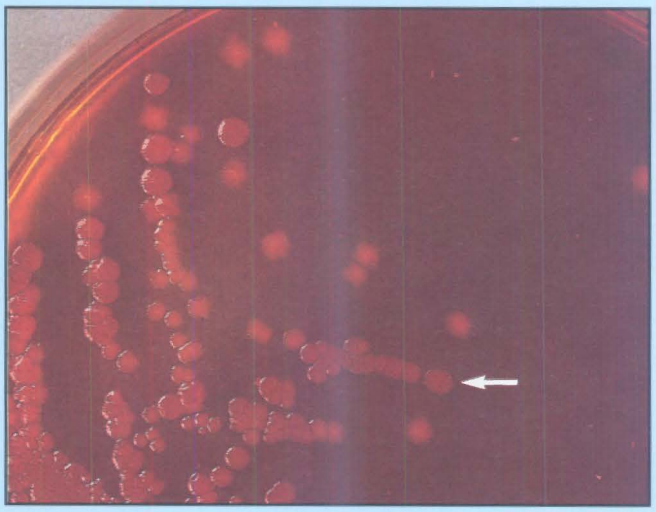
Precautions

Prolonged incubation may lead to confusing results. Do not incubate beyond 48 hours.

Test the medium with a laboratory stock strain of *Shigella species* which is in the R-phase. R-phase shigellae should grow satisfactorily on MacConkey Agar.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
2 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Food.* APHA Inc. Washington DC.
3 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc. Washington DC.
4 Trepeta, A.W. and Edburg, S.C. (1984) *J. Clin. Microbiol.* **19,** 172–174.
5 Maddocks, J.L. and Greenan, M.J. (1975) *J. Clin. Pathol.* **28,** 686–687.
6 Rappaport, F. and Henig, E. (1952) *J. Clin. Path.* **5,** 361.



Appearance of *E. coli* (arrowed) on MacConkey Agar number 3.

Tergitol-7 Agar

Tergitol-7 Agar
Code: CM793

A selective medium for the detection of coliforms, E. coli, Shigella and Salmonella.

Formula

	grams/litre
Peptone	10.0
Yeast extract	6.0
Meat extract	5.0
Lactose	20.0
Bromothymol blue	0.05
Tergitol-7	0.1
Agar	13.0

pH 7.2 ± 0.2

Addition

TTC Solution (SR148) is supplied as 5 ml of filter sterilised 0.05% aqueous solution of tri-phenyltetrazolium chloride (TTC). See Directions for use.

Directions

Suspend 54.15 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense in 100 ml volumes and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and add the contents of 1 ampoule of SR148. Mix well and pour into sterile petri dishes.

Description

Tergitol-7 Agar is a selective and differential medium for the detection and enumeration of coliforms in food and water samples. It may also be used to detect Shigella and Salmonella.

Tergitol-7 Agar is based on the formulation described by Chapman[1] and is recommended for the selective isolation and differentiation of the coliform group. The use of Tergitol-7 as a selective agent had been described earlier.[2]

The addition of tri-phenyltetrazolium chloride (TTC)[3] allows earlier recognition and identification of *Escherichia coli* and *Enterobacter aerogenes*. This medium has been recommended for examining foodstuffs for faecal contamination[4] and has been successfully used in routine water analysis.[5]

The fluorogenic substrate 4-methyl-umbelliferyl β-D-glucuronide (MUG) has been incorporated in Tergitol-7 Agar in a method designed to enumerate *E. coli* in food in 24 hours.[6]

Tergitol-7 inhibits Gram-positive organisms and minimises the swarming of Proteus allowing superior recovery of coliforms. Fermentation of lactose is seen by a change in colour of the pH indicator bromothymol blue. TTC is rapidly reduced to insoluble red formazan by most coliform organisms except *E. coli* and *Enterobacter aerogenes*, thus allowing easy differentiation.

Technique

Inoculate by spreading the sample on the surface of the agar. Incubate at 35°C for up to 24 hours.

Escherichia coli	Yellow colonies with yellow zone. Sometimes with rust coloured centre
Enterobacter/Klebsiella species	Greenish/yellow colonies
Salmonella species	Red colony with bluish zone
Shigella species	Red colony with bluish zone
Proteus species	Red colony with bluish zone
Pseudomonas species	Red colony with bluish zone
Gram-positive bacteria	No growth to slight growth

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Staphylococcus aureus ATCC® 25923

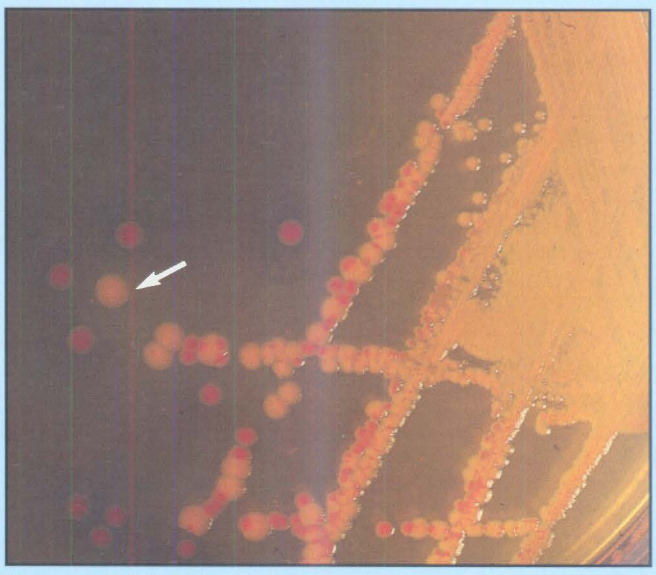
Precautions

Tergitol-7 Agar is designed for early detection of *Esch. coli*, i.e. 6–10 hours incubation.

Incubation at 44°C has been recommended[4,5] for *E. coli* but it may be too high a temperature for some enteropathogenic strains.

References

1 Chapman, G.H. (1947) *J. Bact.* **53,** 504.
2 Pollard, A.L. (1946) *Science* **103,** 758–759.
3 Chapman, G.H. (1951) *Am. J. Pub. Hlth* **41,** 1381.
4 Mossel, D.A.A. (1962) *J. Appl. Bact.* **25,** 20–29.
5 Kulp, W., Mascoli, C. and Tavshanjian, O. (1953) *Am. J. Pub. Hlth* **43,** 1111–1113.
6 Damare, J.M., Campbell, D.F. and Johnston, R.W. (1985) *J. Food Sci.* **50,** 1736–1737 and 1746.



Appearance of *E. coli* (arrowed) on Tergitol-7 Agar.

Chromogenic *Escherichia coli*/Coliform Medium

Chromogenic *Escherichia coli*/Coliform Medium
Code: CM956

A chromogenic medium to aid differentiation between Escherichia coli and other coliforms in cultures produced from food and environmental samples.

Formula

	grams/litre
Chromogenic mix	20.3
Agar	15.0
Yeast extract	3.0
Peptone	5.0
Lactose	2.5
Sodium chloride	5.0
Di-sodium hydrogen phosphate	3.5
Potassium di-hydrogen phosphate	1.5
Neutral red	0.03

pH 6.8 ± 0.2

Directions

Suspend 55.8 g of Chromogenic *Escherichia coli*/Coliform Medium in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile petri dishes.

Description

Escherichia coli is a common Gram-negative microorganism which may be present in food and water samples. The detection of *E. coli* and its differentiation from other coliforms is important in medical and environmental analysis.

The presence of *E. coli* cells in food may be indicative of faecal contamination, although their consumption does not necessarily have adverse effects upon health. Nevertheless, certain strains of *E. coli* are responsible for diarrhoeal disease and can also lead to more serious forms of illness.

Chromogenic *Escherichia coli*/Coliform Medium CM956, is a plating medium which uses two enzyme substrates to improve differentiation between *E. coli* and other coliforms.

One chromogen allows specific detection of *E. coli* through the formation of purple colonies. This substrate is cleaved by the enzyme β-glucuronidase which is produced by approximately 97% of *E. coli* strains.[1]

The other chromogen is cleaved by the enzyme β-galactosidase, which is produced by the majority of coliforms,[1] resulting in rose/pink colonies.

Technique

Dry the surface of the medium. Prepare the food sample by diluting 1 in 5 or 1 in 10 (as appropriate) with 0.1% (w/v) sterile Peptone Water CM9, and homogenise in a stomacher or laboratory blender.

Pipette 0.5 ml or 1.0 ml (as appropriate) of the homogenate onto the plate and spread over the plate surface using a sterile glass spreader. Incubate plates for 18–24 hours at 37°C. Multiply the number of purple colonies by the dilution factor and express the result as the number of *E. coli* per gram of food.

Storage Conditions and Shelf Life

Chromogenic *Escherichia coli*/Coliform Medium CM956 must be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

Quality Control

Positive control:
Escherichia coli ATCC® 25922 – purple colonies
Klebsiella pneumoniae ATCC® 11228 – rose/pink colonies

Negative control:
Pseudomonas aeruginosa ATCC® 27853 – straw colonies

Appearance

Chromogenic *Escherichia coli*/Coliform Medium CM956 is a pale pink powder.

Precautions

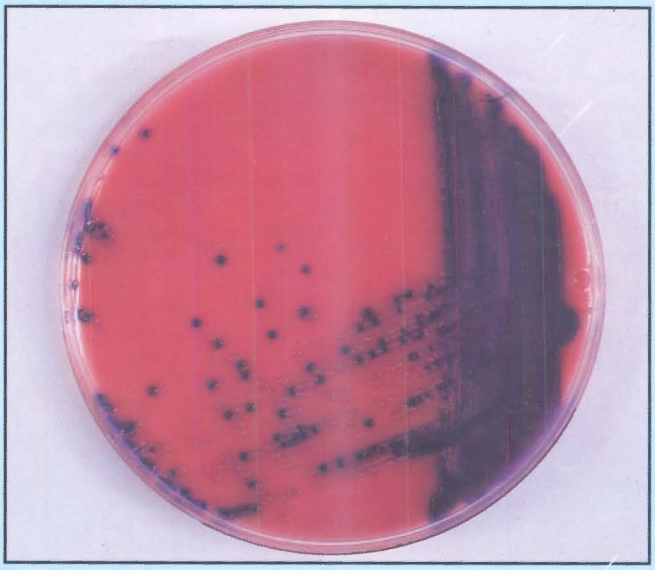
Chromogenic *Escherichia coli*/Coliform Medium CM956 must only be used for *in vitro* diagnostic purposes.

Do not use beyond the stated expiry date, or if the product is caked, discoloured or shows any sign of deterioration.

Wear a dust mask when handling the dehydrated product. Avoid contact with the eyes.

Reference

1 Kilian, M. and Bulow, P. (1976) *Acta. Pathol. Microbiol. Scand. Sect.* **B84,** 245–251.



Appearance of *E. coli* and coliforms on chromogenic *Escherichia coli*/coliform medium. *E. coli*: Purple colonies Coliforms: Pink colonies.
Oxoid Marketing Collection.

Tryptone Bile Agar

Tryptone Bile Agar
Code: CM595

A rapid and direct plate method for the enumeration of Escherichia coli in food.

Formula

	grams/litre
Tryptone	20.0
Bile salts No. 3	1.5
Agar	15.0
pH 7.2 ± 0.2	

Directions

Suspend 36.5 g in 1 litre of distilled water and bring gently to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour 12–15 ml of the medium into sterile dishes.

Description

Tryptone Bile Agar CM595 has been developed according to the formulation of Anderson and Baird-Parker[1] for the detection and enumeration of *Escherichia coli* in foods.

It has several advantages over older methods:

1 It is faster
2 It is less variable
3 It gives better recovery from frozen samples
4 It detects anaerogenic and poor lactose-fermenting strains.

The Direct Plating Method (DPM) described by Anderson and Baird-Parker is a modification of that described by Delaney *et al.*[2] This method, developed for the detection and enumeration of *Esch. coli* in water and food samples, utilises the ability of *Esch. coli* to produce indole from tryptophan at 44°C when grown on a cellulose acetate membrane on plates of Tryptone Bile Agar.

The authors concluded that the formation of indole was a more reliable characteristic for both enterotoxigenic and non-enterotoxigenic strains of *Esch. coli* than lactose fermentation. Ewing[3] found that only 90% of *Escherichia* strains produce acid from lactose within two days, whereas 99% of strains produce indole.

The International Commission on Microbiological Specifications for Foods (CMSF)[4] compared the Most Probable Number (MPN) and the Anderson-Baird-Parker Direct Plating Method (DPM) and concluded that the DPM was preferable to the MPN method of enumeration of *Esch. coli* in raw meats, because of less variability, better recovery from frozen samples, greater rapidity and the smaller quantity of medium needed.

The Direct Plating Method will enumerate both anaerogenic and late lactose-fermenting strains of *Esch. coli* which would be missed by the MPN method. According to Ewing[3] these organisms comprise as many as 10% of *Escherichia* strains.

Holbrook *et al.*[5] have further modified the Direct Plating Method for detection and enumeration of sublethally damaged cells of *Esch. coli* in frozen, dried, heat processed or acid foods. In this modification the inoculum is applied to a cellulose acetate membrane on Minerals Modified Glutamate Agar and incubated for 4 hours at 37°C. The resuscitation step permits the repair of stressed cells before the transfer of the membrane to a Tryptone Bile Agar plate.

It has been shown that the presence of high levels of fermentable carbohydrates will inhibit the synthesis of tryptophanase[6] and thereby stop indole formation. Holbrook *et al.* have demonstrated that the resuscitation step reduces the high concentration of sugar present in the inoculum to a level which does not interfere with the production of indole by *Esch. coli* when grown on Tryptone Bile Agar. The resuscitation step should always be carried out when testing dairy or other products containing high concentrations of sugars.

The indole reagent described by Vracko and Sherris[7] was found to be the most suitable, giving the most distinct reaction and reproducibility. The reagent, 5% p-dimethyl-aminobenzaldehyde in 1N hydrochloric acid is easy to prepare and will not deteriorate when kept for three months in the dark at room temperature.

All indole positive strains give well defined pink colonies when "stained" using the indole reagent; colonies that do not produce indole are straw coloured.

The growth of indole positive organisms other than *Esch. coli* is inhibited by the selective action of the bile salts and the elevated incubation temperature.

The "stained" membranes may be "fixed" by drying in direct sunlight or under a low pressure fluorescent ultra violet lamp with a "Woods" type filter. When dried the intensity of the staining reaction is improved, and such membranes may be stored for reference.

Technique

Direct Plating Method

1 Prepare plates of Tryptone Bile Agar CM595 and dry the surface.
2 Place a cellulose acetate filter membrane (85 mm diameter, 0.45 µ pore size), which need not be sterilised, on the surface of the medium. Gently flatten with a sterile spreader to remove trapped air.
3 Prepare the food sample by diluting 1 in 5 or 1 in 10 with 0.1% (w/v) sterile Peptone Water CM9 and homogenise in a "stomacher" or a laboratory blender.[8]
4 Pipette 0.5 or 1.0 ml of the homogenate onto the membrane and spread over the surface with a sterile glass spreader.
5 Allow the homogenate to soak in and incubate plates stacked, not more than three high, with lids uppermost, for 18–24 hours in a water jacketed incubator at 44°C (±1°).
6 Remove the plates from the incubator and pipette 1–2 ml of the indole reagent into each labelled lid.
7 Lift the membrane with a pair of forceps from the plate and lower onto the reagent.
8 Place the stained membranes in direct sunlight or under a low pressure uv lamp for 5–10 minutes. Indole positive colonies are stained pink.
9 Multiply the number of pink colonies by the dilution factor and express the result as the number of *Esch. coli* per gram of food.
10 The "stained" membrane may be "fixed" by prolonged drying in direct sunlight or under a uv lamp, and kept for reference.

Resuscitation Procedure

1 Preparation of Minerals Modified Glutamate Agar plates. Make up 1 litre of Minerals Modified Glutamate. Medium CM607 and add 12 g of Agar No. 1 L11. Bring gently to the boil until dissolved completely and sterilise by autoclaving at 116°C for 10 minutes. Cool to 50°C and pour 12–15 ml of the medium into sterile dishes.
2 Place a cellulose acetate filter membrane onto the well dried surface of a plate of Minerals Modified Glutamate Agar. Gently flatten with a sterile spreader to remove trapped air.
3 Prepare the food sample by diluting 1 in 5 or 1 in 10 with

0.1% (w/v) Peptone Water CM9 and homogenise in a "stomacher" or a laboratory blender.

4 Pipette 0.5 or 1.0 ml of the homogenate onto the membrane and spread completely over the surface with a sterile glass spreader.

5 Allow the homogenate to soak in, and incubate the plates with the lids uppermost in piles of not more than three for 4 hours at 35°C.

6 Transfer the membrane filter from the plate using sterile forceps and gently lower onto the dried surface of a Tryptone Bile Agar plate.

7 Incubate the plates as described for the Direct Plating Method, stain, and count the number of pink indole positive colonies.

If required the unstained plates may be placed in the refrigerator overnight and the indole test carried out the following morning.

Indole Reagent

5% p-dimethylaminobenzaldehyde in 1N hydrochloric acid.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

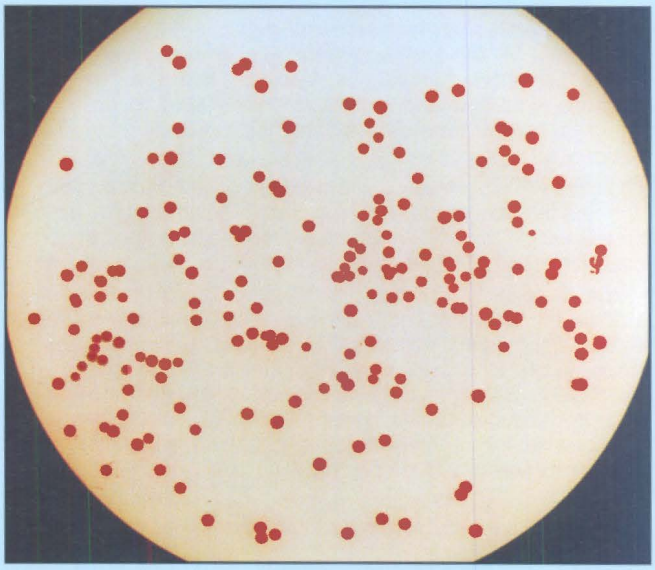
Stain colonies on the membrane filter with indole reagent.

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Enterobacter aerogenes ATCC® 13048

References

1 Anderson, J.M. and Baird-Parker, A.C. (1975) *Appl. Bact.* **39,** 111–117.
2 Delaney, J.E., McCarthy, J.A. and Grasso, R.J. (1962) *Wat. Sewage Works* 109, 289.
3 Ewing, W.H. (1972) *COC Atlanta, US Dept. of Health, Education & Welfare.*
4 International Commission on Microbiological Specifications for Foods (1979) *Can. J. Microbiol.* **25,** 1321–1327.
5 Holbrook, R., Anderson, J.M. and Baird-Parker, A.C. (1980) *Food Technol. in Aust.* **32,** 78–83.
6 Clarke, P.H. and Cowen, S.T. (1952) *J. Gen. Microbiol.* **6,** 187–197.
7 Vracko, R. and Sherris, J.C. (1963) *Amer. J. Clin. Path.* **39,** 429–432.
8 Sharpe, A.N. and Jackson, A.K. (1972) *Appl. Microbiol.* **24,** 175–178.



Appearance, following exposure to indole reagent, of *E. coli* on a filter membrane incubated on Tryptone-Bile Agar.

Oxoid Marketing Collection.

Tryptone Bile X-glucuronide Agar (TBX)

Tryptone Bile X-glucuronide Agar (TBX)
Code: CM945

A medium for the detection and enumeration of E. coli without the need for a filter membrane or indole reagent.

Formula

	grams/litre
Tryptone	20.0
Bile salts No. 3	1.5
Agar	15.0
X-glucuronide	0.075
pH 7.2 ± 0.2	

Directions

Suspend 36.6 g of TBX Medium CM945 in 1 litre of distilled water. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and pour the medium into sterile petri dishes.

Description

TBX Medium is based on Tryptone Bile Agar CM595. Tryptone Bile Agar was originally formulated to improve on earlier methods used to detect *E. coli* in foods[1] in terms of speed, reliability, better recovery from frozen samples and the detection of poor lactose fermenters.

Most *E. coli* strains can be differentiated from other coliforms by the presence of the enzyme glucuronidase. The chromogen in TBX Medium is 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (X-glucuronide), and is targeted by this enzyme. *E. coli* cells are able to absorb this complex intact and intracellular glucuronidase splits the bond between the chromophore and the glucuronide. The released chromophore is coloured and builds up within the cells, causing *E. coli* colonies to be coloured blue/green.

Unlike MUG, where the fluorophore leaches out of the cell into the surrounding agar, the released chromophore in TBX Medium is insoluble and accumulates within the cell. This ensures that coloured target colonies are easy to identify.

Technique

Dry the surface of the medium. Prepare the food sample by diluting 1 in 5 or 1 in 10 (as appropriate) with 0.1% (w/v) sterile Peptone Water CM9 and homogenise in a stomacher or a laboratory blender.

Pipette 0.5 ml or 1.0 ml (as appropriate) of the homogenate onto the plate and spread over the surface with a sterile glass spreader. Incubate plates for 4 hours at 30°C then 18 hours at 44°C.

Multiply the number of blue/green colonies by the dilution factor and express the result as the number of *E. coli* per gram of food.

3–4% of *E. coli* are glucuronidase negative, notably *E. coli* 0157 strains.[2]

Storage Conditions and Shelf Life

TBX Medium CM945 should be stored tightly capped in the original container at 10°C–25°C. When stored as directed, the medium will remain stable until the expiry date printed on the bottle.

TBX Medium CM945 is a light-coloured, free-flowing powder.

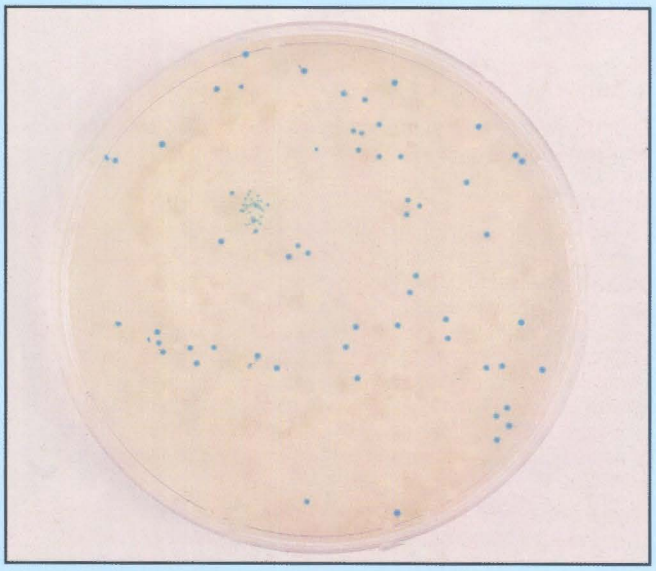
Quality Control

Positive control:
Escherichia coli ATCC® 25922 – blue/green colonies

Negative control:
Klebsiella pneumoniae ATCC® 11228 – colourless colonies

References

1 Anderson, J.M. and Baird-Parker, A.C. (1975) *J. Appl. Bact.* **39,** 111–117.
2 Ratnam, S., March, S.B., Ahmed, R., Bezanson, G.S. and Kasatiya, S. (1988) *J. Clin. Microbiol.* **26,** 2006–2012.



Appearance of *E. coli* colonies on Tryptone-Bile X-glucuronide Agar (TBX Agar). Note that the colour does not diffuse from the colonies.
Oxoid Marketing Collection.

MUG Reagent

MUG Reagent
Code: BR71

A fluorescent reagent for the detection of Escherichia coli.

Vial content

4-methylumbelliferyl-β-D-glucuronide	50 mg

Directions

Add 2 ml of distilled water to a vial and invert gently until completely dissolved. Add the vial contents to the following volumes of suggested media, before sterilisation.

Medium	Final concentration MUG per litre	Number of vials per litre
Violet Red Bile CM107	100 mg	2
MacConkey Agar No. 3 CM115	100 mg	2
Brilliant Green Bile (2%) Broth CM31	50 mg	1
MacConkey Broth Purple CM5a	50 mg	1
Lauryl Tryptose Broth CM451	50 mg	1

Other media can be used; the above concentrations are suggested as a guide but may need adjustment.

Description

Oxoid MUG Reagent is a freeze-dried presentation of the fluorogenic substrate 4-methylumbelliferyl-β-D-glucuronide (MUG). This substrate is hydrolysed by the enzyme glucuronidase to release 4-methylumbelliferone which fluoresces blue/green under ultra-violet light of 366 nm wavelength. The incorporation of MUG into culture media improves the sensitivity and specificity of *E. coli* detection.[1,2,3] Improved sensitivity is mainly due to the detection of anaerogenic strains when present in mixed cultures. The sensitivity for various media has been found to vary from 59% to 85.8%.[4]

Mead, Smith and Williams[5] reported the biosynthesis of the glucuronides of umbelliferone and 4-methylumbelliferone which they subsequently used in fluorometric determination of β-glucuronidase activity. β-glucuronidase was discovered in *E. coli* in 1951[6] but it was over twenty years later that its relative specificity for identification purposes became clear.[7] Since then numerous reports have confirmed its presence in greater than 90% of strains and its scarceness in other members of the Enterobacteriaceae.

Maddocks and Greenan[8] investigated the activity of β-glucuronidase and other enzymes on fluorogenic substrates as faster alternatives to conventional biochemical tests for identifying bacteria. MUG was found to fluoresce well in cultures of *E. coli* while organisms used as controls did not. However, they appear not to have realised the specificity of the enzyme for *E. coli*. Alkaline pH was seen to increase the intensity of fluorescence and in their methodology Maddocks and Greenan used 0.1 N sodium hydroxide to maximise light output. The dependency on pH for fluorescence had been reported by Goodwin and Kavanagh[9] and their observation was confirmed by Freir and Hartman[10] who exposed their membrane filter cultures to ammonia to enhance fluorescence. Wherever possible the pH of growth media containing MUG should be neutral or slightly alkaline. The acidification of the agar surrounding *E. coli* colonies on lactose-based media diminishes the discrimination of MUG-hydrolysing colonies.[11]

The efficacy of MUG is influenced by factors apart from pH including the concentration of the substrate, the type of medium in use, incubation time and incubation temperature. The conditions necessary for optimal use of MUG in solid media for detection and enumeration of *E. coli* in foods have been reported.[12] The conclusions from the study indicate that the use of solid media that do not contain differential substances is preferable and that the choice of incubation conditions and the concentration of MUG may depend on the work undertaken and the level of sensitivity required.

Technique

Follow the method and procedure relevant to the sample and the selected medium. Uninoculated tubes or agar plates should be used as controls (see Precautions).

After incubation detect glucuronidase activity by examining the microbial growth under uv light (366 nm).

The presence of blue/green fluorescence indicates glucuronidase activity.

Report fluorescence as showing the presumptive presence of *Esch. coli* and confirm by further biochemical tests.

Storage and Stability

MUG Reagent should be stored at 2–8°C. When stored as directed the unopened vial is stable until the expiry date on the label.

Quality Control

Positive control:
Escherichia coli ATCC® 25922

Negative control:
Proteus mirabilis ATCC® 110975

Precautions

The presence of endogenous glucuronidase in shellfish samples may result in false positive fluorescence if MUG is incorporated into the presumptive detection medium.[13] This difficulty may be overcome by putting the MUG into the confirmatory medium.

Test tubes used in the MPN method should be checked under uv light to ensure the glass does not fluoresce.

To avoid false positive fluorescence the source of long wave uv light must not exceed 6 watts.

References

References are listed on the following page.

References

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2 Feng, P.C.S. and Hartman, P.A. (1982) *Appl. Environ. Microbiol.* **43,** 1320–1329.
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9 Goodwin, R.H. and Kavanagh, F. (1950) *Arch. Biochem. Biophys.* **27,** 152–173.
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12 Villari, P., Lannuzzo, M. and Torre, I. (1997) *Lett. Appl. Microbiol.* **24,** 286–290.
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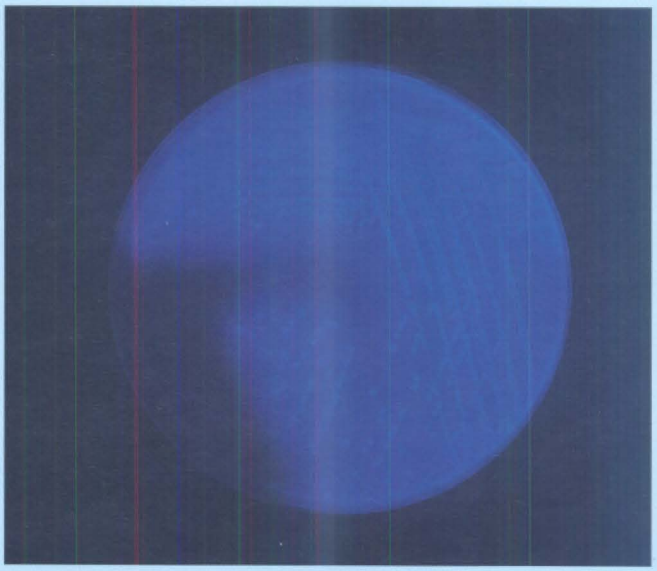
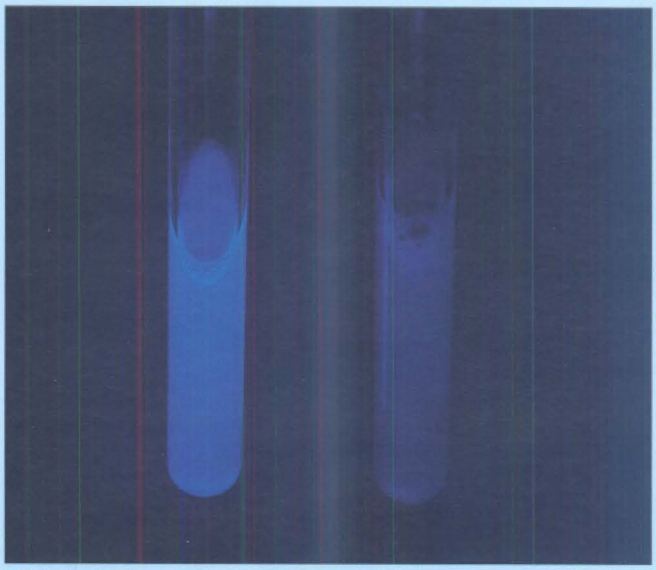


Fluorescing *E. coli* colonies on agar medium containing MUG reagent.
Oxoid Marketing Collection.



Fluorescing *E. coli* growth in broth medium containing MUG reagent. Negative control on right.
Oxoid Marketing Collection.

A brief overview of *Escherichia coli* 0157:H7

Enterohaemorrhagic strains of *E. coli*, and in particular *E. coli* 0157:H7, have emerged as serious enteric pathogens over recent years. The infective dose appears to be very low. Various serogroups including 02, 026, 0104, 0111 and 0145 have been implicated in human disease but 0157 is the most prevalent. The H7 flagella antigen is frequently but not always present on 0157 isolates. It is not unusual for non-motile strains to be isolated.

Enterohaemorrhagic strains are termed verocytotoxigenic because of the characteristic cytopathic effect they have on Vero tissue culture cells.[1]

E. coli 0157 attaches to and alters the surface structure of intestinal mucosal cells and subsequently invades them although the precise workings of the invasive process have still to be established. A toxin virtually identical with that produced by *Shigella dysenteriae* is produced and is responsible for the gastroenteritis which ranges in severity from mild to severe bloody diarrhoea and haemorrhagic colitis. A proportion of cases proceed to develop Haemolytic Uraemic Syndrome (HUS) which is a triad of microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure. Other major organs including the central nervous system, heart and pancreas may also be affected. Death may ensue.

E. coli 0157 is remarkable for the variety of enteric conditions it causes ranging from asymptomatic carriage to life-threatening conditions. The reasons for this are not clear but there are indications that patients infected with strains that produce type 2 verocytotoxin are more likely to develop HUS.

Children are most at risk of developing HUS but the elderly are also affected. The incidence of infection appears generally higher in some geographical locations. At present the reasons for this are not understood.

The main source of *E. coli* 0157 appears to be cattle but other animal species have been implicated.[2] This serogroup is commonly found in pigs but pig strains tend to be enterotoxigenic rather than verocytotoxigenic. Carriage in cattle occurs for long periods but is not permanent. More than one strain may be present at any one time. It appears possible that cattle may on occasions contract the organism from humans, possibly through sewage slurry spread as fertiliser on grazing land. Carriage of the organism by wild birds has been demonstrated, much as transmission of salmonella and Campylobacter by scavenging gulls has been shown, and this may also provide a route for transmission to cattle. The viable but non-culturable state (VNC) has been detected in *E. coli* 0157 and this will complicate detection in the environment.[3] Low temperature appears to be the primary reason for entry into the VNC state.

Human infection is commonly derived from beef products,[4] particularly ground beef in which protection from the lethal effects of cooking may be afforded to the organisms by fat in the meat. Unpasteurised milk and contaminated water have also been responsible.

A variety of other sources of infection have been reported, probably arising from cross-contamination during food storage or contact with bovine manure.

Infected persons may continue to excrete *E. coli* 0157 for several weeks. In this respect the organism is similar to Shigella. Initial studies on excretion suggested that it clears quite rapidly but now these results seem likely to have been due to insensitive methodology.

Person-to-person spread occurs and infection has been contracted following visits to farms. There is a distinct occupational risk for farm and abattoir workers, health professionals caring for infected patients, diagnostic laboratory workers and researchers.

Detection is by direct culture or by selective enrichment followed by plating on Sorbitol MacConkey Agar.[5] Sensitivity is increased by concentrating cells from enrichment culture by immuno-magnetic separation. *E. coli* 0157 does not possess glucuronidase and does not ferment sorbitol. Advantage is taken of these characteristics in formulating culture media for presumptive identification of colonies for further testing. *E. coli* 0157 is also relatively resistant to potassium tellurite and this characteristic is employed in improving the selectivity of Sorbitol MacConkey Agar.

Laboratory culture incubation temperatures above 42°C are not as well tolerated as by other *E. coli* strains.

Infection may also be diagnosed serologically by detection of antibody. This is particularly useful where it has not been possible to culture the organism.

E. coli 0157 is not innately more heat resistant than other *E. coli* serotypes and is in general more heat susceptible than some other pathogens. Minimum temperature for growth is around 6°C under ideal conditions but the maximum temperature tolerated has yet to be clearly determined.

Clear information about the effect of pH on survival is still required but the organism is obviously acid resistant. The effect of acidity varies with the type of acid and is influenced by temperature and the nature of the food or substrate in which the organism is present.

Sodium chloride is a growth-limiting factor but the effective concentration varies with temperature.

E. coli 0157 is not unusually resistant to food preservatives. It is sensitive to benzoate and to ethanol.

E. coli 0157 is no more resistant to the commonly-used disinfectants than other *E. coli*.

Ultimately prevention of infection by *E. coli* 0157 and other enterohaemorrhagic *E. coli* will be through control of food production at source on the farm and during food manufacture and preparation. Preventing the organisms entering the food chain will remain difficult even when the ecology and the ways in which animals contract *E. coli* 0157 is better understood. Application of hazard analysis and critical control point principles by food manufacturers appear likely to be beneficial but, as is necessary with other foodborne pathogens, hygienic kitchen practices and adequate cooking in the domestic environment will continue to be important in preventing infection.

References

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3 Rigsbee, W., Simpson, L.M. and Oliver, J.D. (1997) *J. Food Safety,* **16,** 255–262.
4 Lane, W., Robson, M. and Leurg, A.K.C. (1990) *Postgraduate Med.* **88,** 135–140.
5 *Practical Food Microbiology.* Roberts, D., Hooper, W. and Greenwood, M. (eds.). Public Health Laboratory Service, London 1995.

Table 4 – Media used in some Procedures for Detection of *E. coli* 0157:H7.

Body	Enrichment	Plating
Public Health Laboratory Service[1]	(a) Buffered peptone water with cefixime, cefsulodin and vancomycin	(a) Sorbitol MacConkey Agar
	(b) Tryptone soya broth with bile salts and novobiocin	(b) Sorbitol MacConkey Agar with cefixime and potassium tellurite
University of Wisconsin[2]*	Tryptone soya broth with casamino acids and acriflavine-HCl	Sorbitol MacConkey Agar with MUG**
Campden and Chorleywood Food Research Association[3]	(a) Buffered peptone water with cefixime, cefsulodin and vancomycin	(a) Sorbitol MacConkey Agar
	(b) Modified EC broth with novobiocin	(b) Sorbitol MacConkey Agar with cefixime and potassium tellurite
		(c) Violet Red Bile Agar with MUG**
FDA[4]	EHEC Enrichment broth (Tryptone soya broth with cefixime, cefsulodin and vancomycin)	(a) Sorbitol MacConkey Agar
		(b) Sorbitol MacConkey Agar with cefixime and potassium tellurite
		(c) Haemorrhagic Colitis (HC) Agar

*Confirmation of the presence of *E. coli* 0157:H7 is made by applying the enrichment culture to an ELISA assay using a monoclonal antibody specific for *E. coli* 0157:H7 and 026:H11.
**4 Methylumbelliferyl-β-D-glucuronide.

References

1 *Practical Food Microbiology* (1995) Roberts, D., Hooper, W.H. and Greenwood, M. (eds.). Public Health Laboratory Service.
2 Padhye, N.V. and Doyle, M.P. (1991) *Appl. Environ. Microbiol.* **57,** 2693–2698.
3 Campden and Chorleywood Food Research Association. Technical Manual number 43. Manual of Microbiological Methods for the Food and Drinks Industry. 2nd Edition, October 1995.
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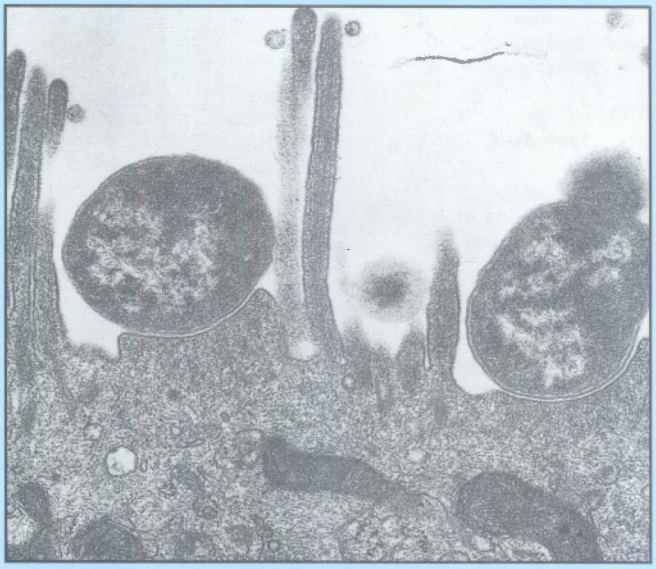
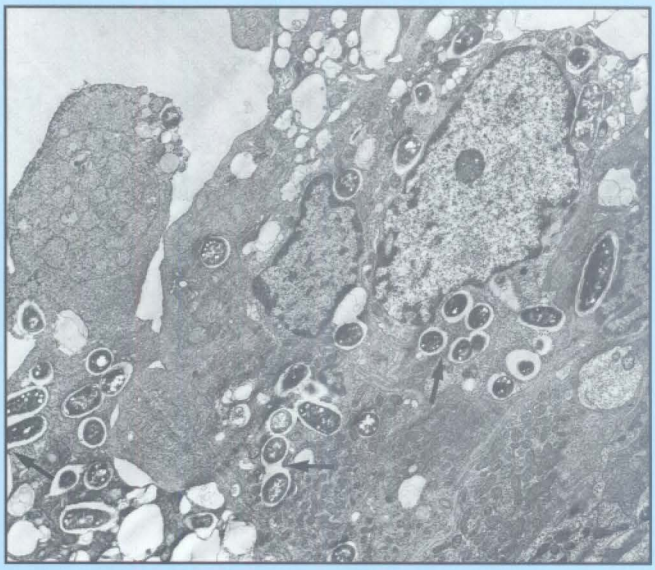


Enteropathogenic *E. coli* (EPEC) cells attached to the small intestinal mucosa. Note the localised destruction (effacement) of the microvilli.

Photograph supplied by Dr Stuart Knutton, Institute of Child Health, University of Birmingham, UK.

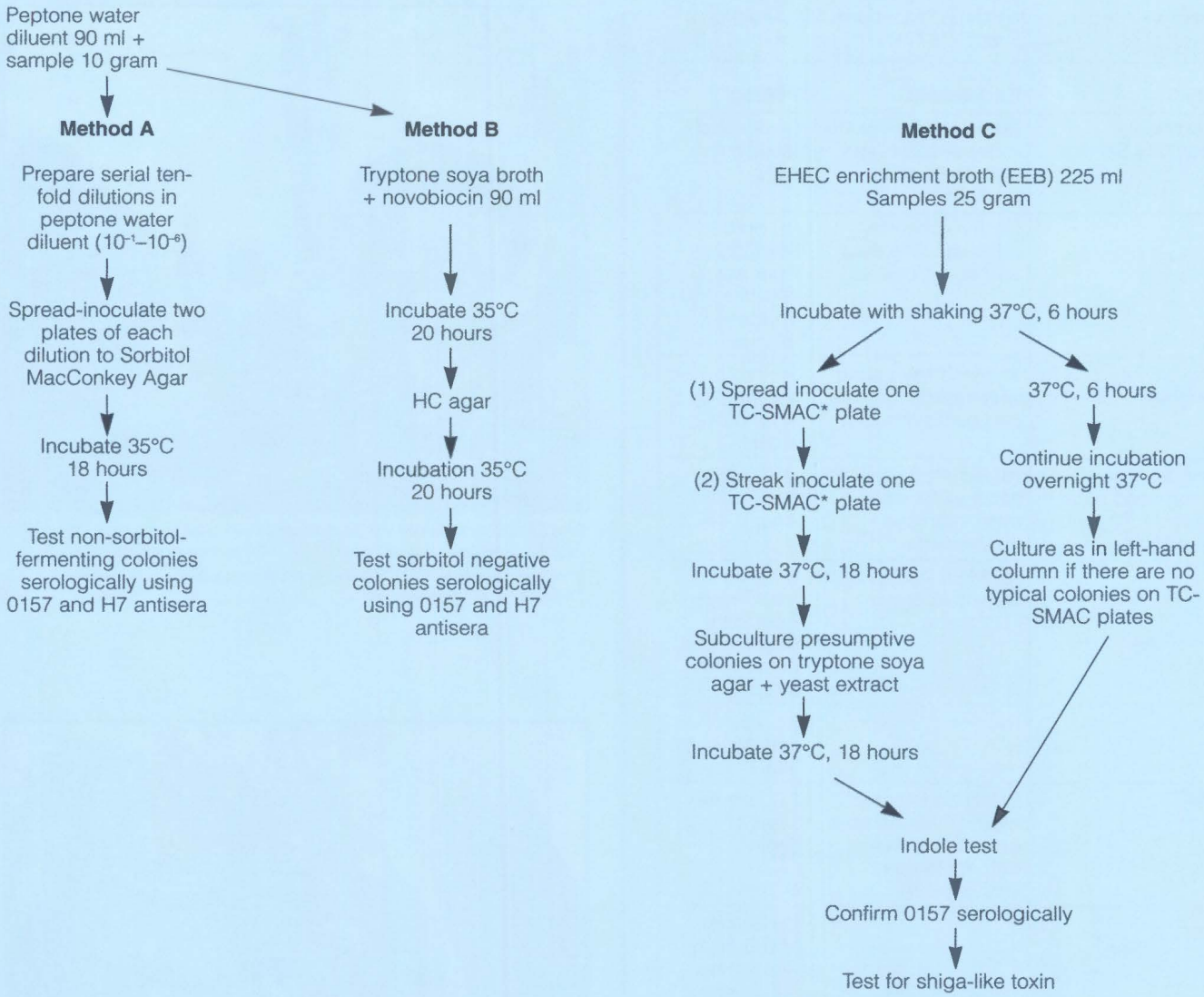


Colonic mucosa infected with enteroinvasive *E. coli* (EIEC). Bacteria, (arrowed) are present inside colonic epithelial cells.

Photograph supplied by Dr Stuart Knutton, Institute of Child Health, University of Birmingham, UK.

A description of EPEC and EIEC strains of *E. coli* is given on pages 5 and 6. Attachment and effacement by enterohaemorrhagic (EHEC) strains has a similar appearance.

FDA-BAM methods for isolation of enterohaemorrhagic *E. coli* (EHEC)



*TC-SMAC: Sorbitol MacConkey Agar containing potassium tellurite 2.5 mgm/litre, cefixime 0.05 mgm/litre.

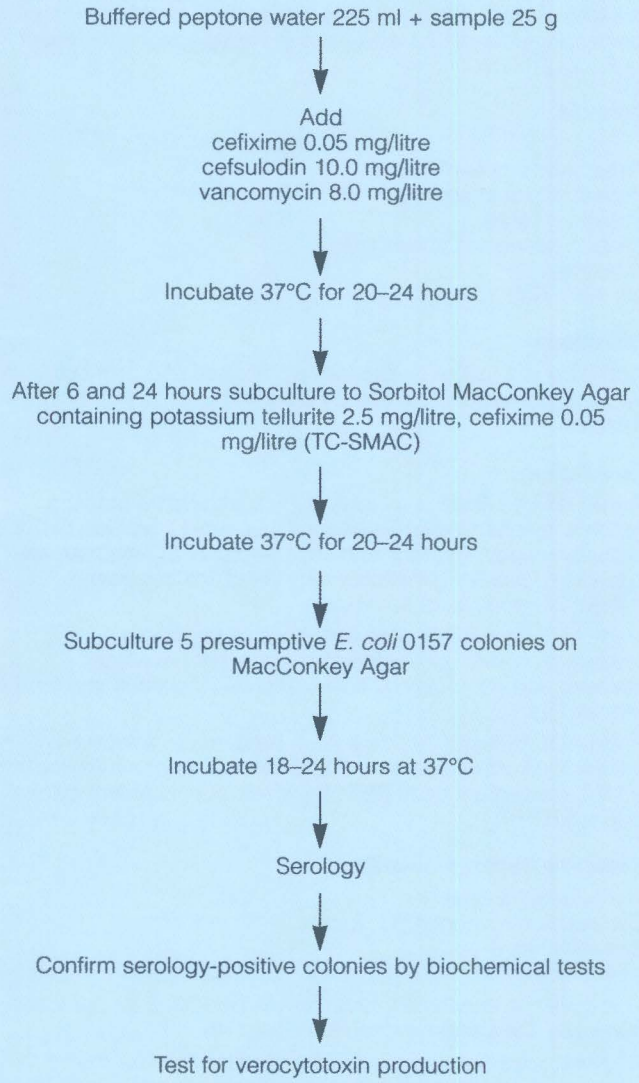
This is an abbreviated version of the procedures used. Microbiologists intending to follow this method should consult the FDA Bacteriological Analytical Manual for full details.

Reference

FDA Bacteriological Analytical Manual, 8th Edition 1995, Chapter 4, AOAC International, Arlington Va.

Public Health Laboratory Service Method 1

Public Health Laboratory Service Method 1 for isolation of *E. coli* 0157:H7 from foods

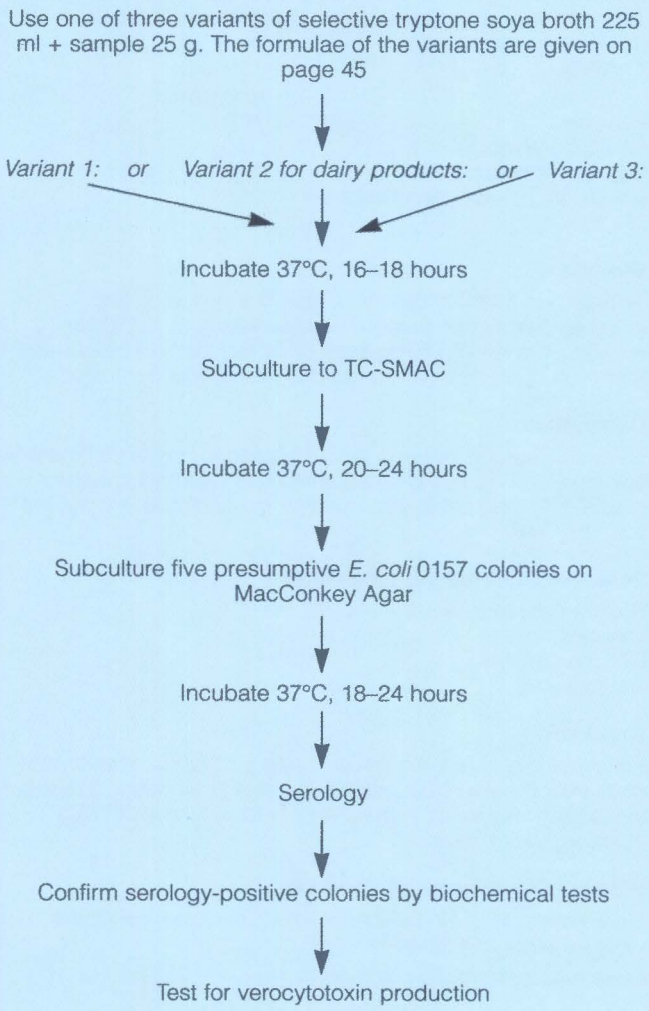


Reference

Practical Food Microbiology (1995) Section 6. Roberts, D., Hooper, W. and Greenwood, M. (eds). Public Health Laboratory Service, London.

Public Health Laboratory Service Method 2

Public Health Laboratory Service Method 2 for isolation of *E. coli* 0157:H7 from foods



Reference

Practical Food Microbiology, Section 6. Roberts, D., Hooper, W. and Greenwood, M. (eds). Public Health Laboratory Service, London 1995.

Buffered Peptone Water

Buffered Peptone Water
Code: CM509

A pre-enrichment medium to be used prior to the selective enrichment of pathogenic Enterobacteriaceae in foods.

Formula

	grams/litre
Peptone	10.0
Sodium chloride	5.0
Disodium phosphate	3.5
Potassium dihydrogen phosphate	1.5

pH 7.2 ± 0.2

Directions

Add 20 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes. It is extremely important that the distilled water used is of high quality with a low mineral content/conductivity.

Description

Buffered Peptone Water is primarily used for pre-enrichment of salmonella in foods before subculture to selective enrichment media. The medium is also used for the selective enrichment of *E. coli* 0157.[1]

Selective Buffered Peptone Water

Buffered peptone water	1000 ml
Cefixime	0.05 mg
Cefsulodin	10.0 mg
Vancomycin	8.0 mg

Caution:

Enrichment cultures should be plated on Sorbitol MacConkey Agar after 6 hours incubation and again at 24 hours. Extended incubation may result in the overgrowth of *E. coli* 0157 by other organisms present.

Storage Conditions and Shelf Life

Store deyhdrated medium below 25°C and use before the expiry date on the label.

Store prepared medium at 2–8°C.

Quality Control

Positive control:
Escherichia coli NCTC 12900. This strain is reported not to produce verocytotoxin

Negative control:
Uninoculated medium

Reference

1 *Practical Food Microbiology*. Roberts, D., Hooper, W. and Greenwood. M. (eds). Public Health Laboratory Service 1995.

Tryptone Soya Broth Soybean Casein Digest Medium USP

Tryptone Soya Broth
Soybean Casein Digest Medium USP
Code: CM129

A highly nutritious general purpose medium for the growth of bacteria and fungi. It is also used in the selective enrichment of E. coli 0157:H7.

Formula

	grams/litre
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Dibasic potassium phosphate	2.5
Glucose	2.5

pH 7.3 ± 0.2

Directions

Add 30 g to 1 litre of distilled water, mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Tryptone Soya Broth is a highly nutritious versatile medium which is recommended for general laboratory use. Due to the inclusion of both tryptone and soya peptone, the medium will support a luxuriant growth of many fastidious organisms without the addition of serum, etc.

Tryptone Soya Broth is also very suitable for use as a resuscitation medium for microbial cells that have been stressed and damaged by environmental influences and food processing.

Phosphate-buffered Tryptone Soya Broth made selective by the addition of bile salts No. 3 and novobiocin or acriflavine (EHEC enrichment broth (EEB)) is used for enrichment culture of *E. coli* 0157.[1]

Selective Tryptone Soya Broth

Tryptone Soya Broth	1000 ml
Dipotassium hydrogen phosphate	1.5 gm
Bile salts No. 3	1.5 gm
Novobiocin	20 mgm

This selective enrichment broth may be modified for particular purposes. Details are available on page 45.

Tryptone Soya Broth is a recommended medium for the recovery of organisms in pharmaceutical products after sterilisation.

Storage Conditions and Shelf Life

Store the deyhdrated medium below 25°C and use before the expiry date on the label.

Store prepared medium at room temperature.

Quality Control

Positive control:
Streptococcus pneumoniae ATCC® 6303
Staphylococcus aureus ATCC® 25923
Escherichia coli NCTC 12900
This strain is reported not to produce verocytotoxin.

Negative control:,
Uninoculated medium

Reference

1 *Practical Food Microbiology*. Roberts, D., Hooper, W. and Greenwood. M. (eds). Public Health Laboratory Service 1995.

Formulae of selective enrichment media for *E.coli* 0157:H7.

1 EHEC enrichment broth (EEB)

	grams/litre
Tryptone Soya Broth	30.0
Bile salts No. 3	1.5
Dipotassium phosphate	1.5
Novobiocin	20.0 mgm
Water	1000 ml
pH 7.4	

Reference

Weagent, S.D., Bryant, J.L. and Jinneman, K.G. (1995). *J. Food Prot.* **58,** 7–12.

2 EC – Novobiocin broth

	grams/litre
Tryptone	20.0
Bile salts No. 3	1.12
Lactose	5.0
Dipotassium hydrogen phosphate	1.5
Potassium dihydrogen phosphate	1.5
Sodium chloride	5.0
Novobiocin	20.0 mgm
Water	1000 ml
pH 6.9 ± 0.1	

Reference

Okrend, A.J.G., Rose, B.E. and Matner, R. (1990). *J. Food Prot.* **53,** 936–940.

Modified tryptone soya broths

3 Variant 1

	grams/litre
Tryptone Soya Broth	30.0
Bile salts No. 3	1.5
Dipotassium hydrogen phosphate	1.5
Novobiocin	20.0 mgm
Water	1000 ml

Variant 2 (for dairy products)

	grams/litre
Tryptone Soya Broth	30.0
Bile salts No. 3	1.5
Potassium dihydrogen phosphate	1.35
Disodium hydrogen phosphate	12.0
Novobiocin	20.0 mgm
Water	1000 ml

Variant 3 (alternative to variant 1)

	grams/litre
Tryptone Soya Broth	30.0
Bile salts No. 3	1.5
Dipotassium hydrogen phosphate	1.5
Casamino acids	10.0
Acriflavine hydrochloride	10.0 mgm
Water	1000 ml

Reference

Practical Food Microbiology, Roberts, D., Hooper, W. and Greenwood, M. (eds). Public Health Laboratory Service, London 1995.

4 Selective Buffered Peptone Water

	grams/litre
Buffered peptone water	20.0
Cefixime	0.05 mgm
Cefsulodin	10.0
Vancomycin	8.0
Water	1000 ml

Reference

Chapman, P.A., Siddons, C.A., Wright, D.J. *et al.* (1993). *Epidemiol. Inf.* **111,** 439–447, cited in *Practical Food Microbiology*, Roberts, D., Hooper, W. and Greenwood, M. (eds). Public Health Laboratory Service, London 1995.

HC Isolation Agar for *E. coli* 0157:H7

	grams/litre
Tryptone	20.0
Bile salts No. 3	1.12
Sodium chloride	5.0
4-methylumbelliferyl-β-D-glucuronide (MUG)	0.10
Bromocresol purple	0.015
Water	1000 ml
pH not specified	

A description of HC Agar can be found on page 20.

Sorbitol MacConkey Agar

Sorbitol MacConkey Agar
Code: CM813

A selective and differential medium for the detection of Escherichia coli 0157 and other non-sorbitol-fermenting enterohaemorrhagic serotypes.

Formula

	grams/litre
Peptone	20.0
Sorbitol	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0
pH 7.1 ± 0.2	

Directions

Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

Sorbitol MacConkey Agar is based on the formulation described by Rappaport and Henig,[1] and is recommended for the isolation of pathogenic *Esch. coli* 0157. Other enterohaemorrhagic non-sorbitol-fermenting serotypes may also be isolated on this medium. The formulation is identical to MacConkey Agar No. 3 except that lactose has been replaced with sorbitol. *Esch. coli* 0157 does not ferment sorbitol and therefore produces colourless colonies. In contrast, most *Esch. coli* strains ferment sorbitol and form pink colonies. The efficiency of Sorbitol MacConkey Agar has been confirmed by March and Ratnam.[2] These workers reported that the detection of *Esch. coli* 0157 on this medium had a sensitivity of 100% and a specificity of 85%. They recommended the medium as a simple, inexpensive, rapid and reliable means of screening *Esch. coli* 0157.[3,4,5,6,7]

Esch. coli 0157 is a cause of haemorrhagic colitis, an illness characterised by bloody diarrhoea and severe abdominal pain. *Esch. coli* and haemorrhagic colitis are linked with Haemolytic Uraemic Syndrome (HUS).

Technique

1 Make up the agar according to the directions and pour into petri dishes. If necessary dry the surface of the agar.

2 Inoculate the plates with a suspension of the food, faeces, etc. to produce separated colonies.

3 Incubate at 35°C for 24 hours. Doyle and Schoeni[8] have reported that 35–37°C is the optimal temperature for growth of *Esch. coli* 0157. At 44 to 45.5°C this *Esch. coli* serotype does not grow well even after 48 hours incubation.

Delay in reading plates beyond 24 hours should be avoided because the colour intensity of sorbitol-fermenting colonies fades, reducing the contrast with non-fermenting colonies.

Other Gram-negative organisms including *Pseudomonas*, *Proteus* and *Klebsiella* species are able to grow on Sorbitol MacConkey Agar but may generally be differentiated by the appearance of their colonies.

The selectivity of the medium can be improved considerably by the inclusion of cefixime and potassium tellurite.[9] (See page 47.)

A diagnostic reagent, *Escherichia coli* 0157 latex test DR620, is available so that instant confirmatory tests can be made from suspicious colonies. See page 48.

Colonial Morphology

Esch. coli 0157 will form colourless but otherwise typical *Esch. coli* colonies.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Escherichia coli 0157 (A non-toxigenic strain is preferred but see precautions).

Negative control:
Escherichia coli ATCC® 25922

Precautions

Although the great majority of *Esch. coli* 0157 strains have a typical appearance on Sorbitol MacConkey Agar, some strains are atypical.[10]

Sorbitol MacConkey Agar cannot be used solely to detect VTEC strains of *Esch. coli* as some non-toxic strains will not ferment sorbitol.[11]

References

1 Rappaport, F. and Henig, E. (1952) *J. Clin. Path.* **5,** 361.
2 March, S.B. and Ratnam, S. (1986) *J. Clin. Microbiol.* **23,** 869–872.
3 Centers for Disease Control 1985 – United States, 1984, *Morbid Mortal Weekly Rep.* **34,** 20–21.
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5 Karmali, M.A., Steele, B.T., Petric, M. and Lim, C. (1983) *Lancet* **i,** 619–620.
6 Pai, C.H., Gordon, R., Sims, H.V. and Bryant, L.E. (1984) *Ann. Intern. Med.* 738–742.
7 Waters, J.R. (1985) *Can. Dis. Weekly Rep.* **11,** 123–124.
8 Doyle, M.P. and Schoeni, S.L. (1984) *Appl. and Envir. Microbiol.* **48,** 855–856.
9 Zadik, P.M., Chapman, P.A. and Siddons, C.A. (1993) *J. Med. Microbiol.* **39,** 155–158.
10 Karmali, M.A. (1988) *Culture* **9,** 2.
11 Lior, H. and Borcryk, A. (1987) *Lancet* **i,** 333.

Cefixime Tellurite Selective Supplement
Code: SR172E

A freeze-dried selective supplement for the isolation of Escherichia coli 0157.

Contents

The box contains 10 vials. Each vial is sufficient to supplement 500 ml of Sorbitol MacConkey Agar (CM813).

Vial contents

Potassium tellurite	1.25 mg
Cefixime	0.025 mg

Directions

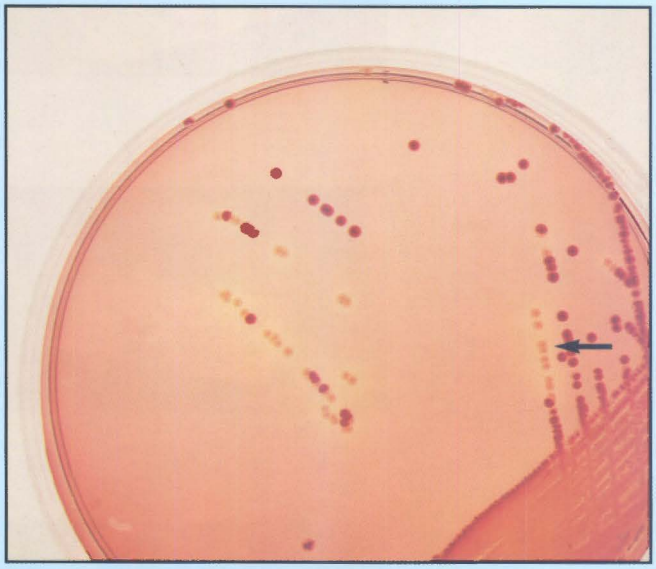
Aseptically add 2 ml of sterile distilled water to one vial and invert gently to dissolve. Aseptically add the vial contents to 500 ml of sterile Sorbitol MacConkey Agar (CM813), cooled to 50°C. Mix well and pour into sterile petri dishes.

Precautions

Cefixime Tellurite Selective Supplement should be used for *in vitro* purposes only. Do not use beyond the stated expiry date.

Storage

Cefixime Tellurite Selective Supplement should be stored below 0°C in the dark. When stored as directed the reagents remain stable until the expiry date shown on the label.



A mixed growth of non-sorbitol-fermenting (arrowed) and sorbitol-fermenting strains of *E. coli* on Sorbitol MacConkey Agar.

E. coli 0157 Latex Test

E. coli 0157 Latex Test
Code: DR620

A highly sensitive and specific latex agglutination test for the identification of E. coli serogroup 0157.

Principle

Blue latex particles are sensitised with specific rabbit antibody reactive with the *Esch. coli* 0157 somatic antigen.

Agglutination will occur in the presence of *Esch. coli* serogroup 0157 forming a lattice which is clearly visible as a positive result.

Procedure

Sorbitol MacConkey Agar should be used as the primary screen. Non-sorbitol-fermenting colonies should then be checked using the following procedure.

Features and Performance

The use of blue latex on disposable white reaction cards ensures that results are easily seen and interpreted. The bottles in the kit have colour-coded caps for easy recognition of positive and negative controls.

The *E. coli* 0157 latex test is very accurate, showing a sensitivity of 100% and specificity of 99%.

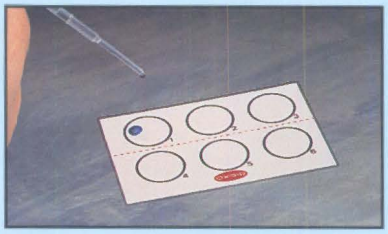
In a study in which the Oxoid latex slide test was compared against the tube agglutination test, Chapman[1] confirmed the very high sensitivity of the latex test. All *E. coli* previously confirmed as 0157 agglutinated in both the latex and tube tests, while all other organisms tested, including *Escherichia hermanii* were negative by both procedures. *E. hermanii* has occasionally been misidentified as *E. coli* by latex agglutination.[2] A separate study has shown the value in the Oxoid kit, of including a latex control to detect false-positive reactions.[3]

References

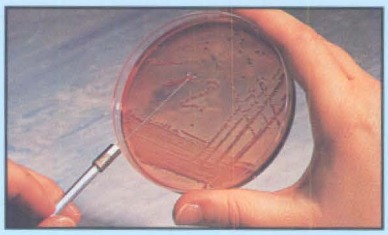
1 Chapman, P.A. (1989) *J. Clin. Pathol.* **42,** 1109–1110.
2 Lior, H. and Borczyk, A.A. (1987) *Lancet* **i,** 333.
3 Borczyk, A.A., Harnett, N., Lombos, M. and Lior, H. (1990) *Lancet*, 336, 946–947.

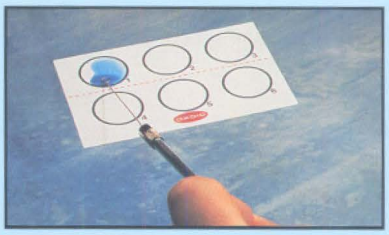
1 Shake Test Reagent vigorously and dispense one drop onto test circle.

2 Add one drop of saline to test circle, ensuring it does not mix with Test Reagent.

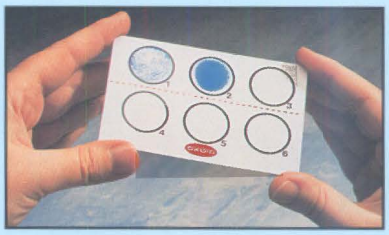


3 Using a sterile loop select a portion of the colony to be tested and emulsify in saline drop.

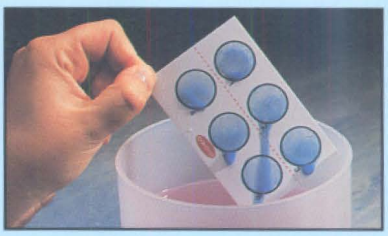




4 Mix Test Reagent and Suspension.



5 Rock reaction card in a circular motion for up to one minute and observe for agglutination. Repeat procedure with other NSF colonies or Control Latex Reagent where appropriate.



6 On completion of tests, dispose of reaction card safely into disinfectant.

An Overview of *E. coli* Toxins

Toxins as virulence factors of *E. coli* virotypes

Possession of adhesins which bind *E. coli* cells to the host cell mucosa and the ability to produce toxins which affect the physiology of host cells are the factors which decide whether a strain of *E. coli* is pathogenic.

Adhesins vary considerably in detailed structure but all are basically similar in being fine rod-like projections from the cell surface. These projections are termed pili or fimbriae.

E. coli produces a number of different toxins. Whether or not they are produced by particular strains and the characteristics of the toxins that are produced are a major discriminating feature used in separating strains into virotypes. Although different virotypes possess different toxins, distinction is not complete as the same toxin may be shared by different virotypes.

Toxin activity is complex and different toxins cause different effects. One effect is stimulation of host cell fluid secretory mechanisms. Impairment of fluid uptake by intestinal mucosal cells and disruption of cell function signalling systems are other mechanisms which result in imbalance of fluid absorption and secretion leading to diarrhoea.

Some strains of *E. coli* invade host cells but invasion is not a prerequisite for toxin activity in all virotypes.

Enterotoxigenic (ETEC) strains resemble *Vibrio cholerae* in that they adhere to the small intestinal mucosa but do not invade. The toxins they produce in close proximity to the mucosal cells apparently gain access to the cell receptors following leakage from the bacterial cell resulting from exposure to concentrations of bile that are normal in the intestine. This leakage may be assisted by the presence of trypsin and the low iron content of the small intestine.

Enteroaggregative (EAggEC) strains are a cause of persistent diarrhoea in children. One of the toxins they produce is similar to the heat-stable toxin of ETEC strains and they also produce a lysin which causes pores to form in the host cell membrane. The pores apparently provide access to the interior of the host cell for passage of calcium ions into the cytoplasm.

The function of the toxins of EAggEC strains in human disease have yet to be fully established.

Enteropathogenic (EPEC) *E. coli* possess no obvious diarrhoeal toxins. Their diarrhoeagenic activity may instead be due to loss of absorptive capacity by mucosal cells caused through damage to the cell surfaces by bacterial cells binding to them, destroying villi in the process of cell effacement. Binding has an effect on the intracellular calcium content, the cytoplasmic content increasing in amount.

Unlike EAggEC strains in which free calcium content in the cell is due to intake from the extracellular environment, the increased free calcium content of EPEC strains is derived from intracellular stores.

In both cases the effect of increased calcium content is essentially the same, leading through an intermediate series of effects to change in host mucosal cell structure and interference with the chemistry involved in signalling of cell function. One consequence of deformation of host cell structure by EPEC strains is that *E. coli* cells may become surrounded by phagocytic vesicles although it is still unclear whether EPEC strains actually invade the mucosa in disease.

Enterohaemorrhagic (EHEC) strains behave very like EPEC strains in attaching tightly to the host mucosal surface and altering cell structure by effacement. They differ from EPEC strains in producing a toxin almost identical to *Shigella shiga* toxin which appears to be responsible for the haemorrhagic colitis and Haemolytic Uraemic Syndrome (HUS) characteristic of EHEC strains.

Enteroinvasive (EIEC) strains cause a disease very similar in its symptoms to bacterial dysentery. They closely resemble *Shigella spp.* in their capacity to invade colon mucosal cells followed by lateral cell-to-cell spread but differ importantly in not producing Shiga toxin which might explain why they do not cause HUS. Production of toxin of any sort is not a feature of these strains. Much detail has yet to be learnt about the virulence factors of EIEC strains but sufficient is already known to indicate that they and the mechanisms which regulate them will be very similar to those of *Shigella spp.*

The Toxins of *E. coli*

1 ST and LT Toxins

Strains of the ETEC virotype produce two major toxins which act on the intestinal epithelium to stimulate fluid secretion. One, Heat Stable Toxin (ST) retains toxin activity after exposure to 100°C for 30 minutes. The second, Heat Labile Toxin (LT) loses toxin activity under these conditions. ST toxin is composed of a family of similar toxins which fall into two subgroups according to whether or not they are soluble in methanol. All are proteins of unusually small molecular size which are inactivated by heat far less readily than larger proteins. They are excreted by the bacterial cells in a sequence that step-wise shortens the peptide length.

LT toxins are considerably larger than ST toxins. There are two subgroups, LT1 and LT2. In its molecular structure and activity LT1 is very similar to cholera toxin. The gene for *E. coli* LT toxin is present on a plasmid and may be transferred to other Gram-negative organisms by conjugation.

LT2 toxin has the same basic structure and mechanism as LT1 toxin and is particularly associated with animal strains.

EAggEC strains of *E. coli* appear not to produce an LT toxin but do produce an ST-like toxin which has been named enteroaggregative ST toxin (EAST).

2 Cytolysin and Haemolysins

EAggEC strains may also produce a cytolysin similar to a haemolysin produced by strains of *E. coli* found in urinary tract infections. This lysin produced by EAggEC strains is not haemolytic but does cause pores to be formed in host cells.

An enterohaemolysin related to, but distinct from, the α-haemolysin produced by faecal and extra-intestinal isolates has been known for some years. Unlike α-haemolysin it is active only if the erythrocytes used to detect it are washed free of other blood components. It is usual to employ sheep cells but human, bovine and guinea-pig cells can also be used.

Haemolysis occurs considerably more slowly than the haemolysis caused by α-haemolysin and is less intense in appearance.

Enterohaemolysin was first found in *E. coli* 026 and 0111 serotypes. These serotypes contain strains that produce Shiga-like toxin and a later study of a variety of strains capable of producing Shiga-like toxin showed that there is a close relationship between the presence of enterohaemolysin and Shiga-like toxin. Subsequently the production of enterohaemolysin has been found to be a useful marker for screening enterohaemorrhagic strains. However, false positive and false negative indications occur and a test for verocytotoxin cannot be dispensed with entirely.

3 Cytolethal Distending Toxin

Some strains of *E. coli* have recently been recognised to share with Campylobacter and Shigella the ability to produce a novel toxin. This toxin is named cytolethal distending toxin

(CLDT) because of the characteristic changes in appearance it produces in a variety of tissue culture cell lines including Vero cells. The toxin causes elongation of the cells followed by distension.

CLDT is different from any other toxin produced by enteric bacteria. It is destroyed by heating at 70°C for 15 minutes and by trypsin.

Inoculation into rabbit ileal loops causes tissue changes typical of an enterotoxic effect but there is no accumulation of fluid.

CLDT has been found in EPEC strains, mostly of serotype 0127:H. It has also been found in EAggEC strains.

The diarrhoeagenic capabilities of the toxin are not yet fully known and it may be that its acts only as an additional virulence factor of diarrhoea.

4 Verocytotoxin (Shiga-like toxin)

Enterohaemorrhagic strains produce toxin that is virtually identical to the Shiga toxin produced by *Shigella dysenteriae*. Both toxins produce a characteristic cytopathic effect on Vero cells and are termed verocytotoxigenic toxin (VT). HeLa cells are also effected. The toxin has also been named Shiga-like toxin (SLT) and the two names are interchangeable.

VT/SLT is divided into four subgroups, VT1 or SLT1, VT2 or SLT2, VT2c or SLT IIc and VT2e or SLT IIe.
Enterohaemorrhagic strains produce VT1 toxin or VT2 toxin or both, indicative that both toxins are involved in pathogenesis.

The first three subgroups are involved in disease of humans and the fourth a disease of pigs, Edema Disease. *Shigella shiga* toxin and *E. coli* Shiga-like toxin are closely related to a group of plant toxins that includes ricin and are part of a family of structurally-related toxins which includes cholera toxin and *E. coli* LT toxin.

Toxin production appears to be regulated by iron concentration and the presence of mucus in the small intestine.

Detection of *E. coli* toxins

Classically, tests to detect toxins have been performed on animals but with increasing rapidity these are being replaced by other systems. Change is occurring because of technological advance, greater public objection to animal experimentation, high cost, impracticality and difficulty in quantifying results from animal tests.

These older, traditional methods include the rabbit ligated loop reaction for LT toxin and the infant mouse test for ST toxin. The appearance of positive reactions in these tests is shown in Tables 1 and 2.

Tests to demonstrate the effects of toxins on animal organs have largely been replaced by the use of cultured mammalian cells. Cells derived from a variety of species are used. These include Chinese hamster ovary cells, (CHo) Y-1 adrenal cells and intestinal epithelial cells for LT toxin, and Vero cells from the kidneys of African green monkeys for verocytotoxin.

The effect of verocytotoxin on Vero cells is shown on page 51.

Tissue culture cell methods, although preferable to the use of animals, are themselves less convenient than methods that dispense entirely with the necessity for living cells. Current commonly-used methods utilise immunological reactions, but it is not unusual for the results obtained from methods using tissue culture to be taken as the Gold Standard against which the performance of immunological methods is judged.

The newest methods, not yet in wide common use, utilise the polymerase chain reaction (PCR) to detect genes responsible for toxin production. A considerable advantage of PCR is that the potential to produce toxin possessed by a strain is detected even if the organism is not producing toxin at the time.

This brief survey of tests to detect toxins of *E. coli* is limited to immunological methods.

A number of immunological methods have been described. Not surprisingly, those for which commercially-produced systems are available have tended to become more commonly used.

Tests described for LT toxin include passive immune haemolysis,[1] the Biken gel diffusion test,[2] derived from the Elek test generally used for identifying toxigenic strains of *Corynebacterium diphtheriae*, radioimmunoassay,[3] enzyme-linked immunosorbent assay (ELISA)[4] and reversed passive latex agglutination (RPLA), used originally for detection of Staphylococcal enterotoxin.[5]

ELISA tests are available for detection of ST and verocytotoxins.

ELISA and RPLA tests that enable verocytotoxin to be detected will enable verocytotoxigenic strains of *E. coli* that are not 0157:H7 to be found.

Oxoid products using latex agglutination and enzyme immunoassay are described on the following pages.

Enterotoxin production by *E. coli* is affected by environmental conditions. Important influences include the composition and pH of culture media, incubation time and temperature, aeration and agitation during incubation, presence of antibiotics and age of the culture. Detailed information about these influences is contained in references cited by Kornacki and Marth in a review of foodborne illness caused by *E. coli*.[6]

A close association between the production of verocytotoxin and enterohaemolysin has been observed. Although the association is not complete, it is close enough for enterohaemolysin production to be a useful marker of verocytotoxigenic strains when investigating mixed cultures. Enterohaemolysin production is detected by culturing on specially prepared plates of nutrient medium containing washed sheep erythrocytes. These plates are available commercially, ready to use.

References

1 Tsukamoto, T., Kinoshita, Y., Taga, S. *et al.* (1980) *J. Clin. Microbiol.* **12,** 768–771.
2 Honda, T., Taga, S., Takeda, Y. and Miwatani, T. (1981) *J. Clin. Microbiol.* **13,** 1–5.
3 Greenberg, H.B., Sack, D.A., Rodriquez, W. *et al.* (1977) *Infect. Immun.* **17,** 541–545.
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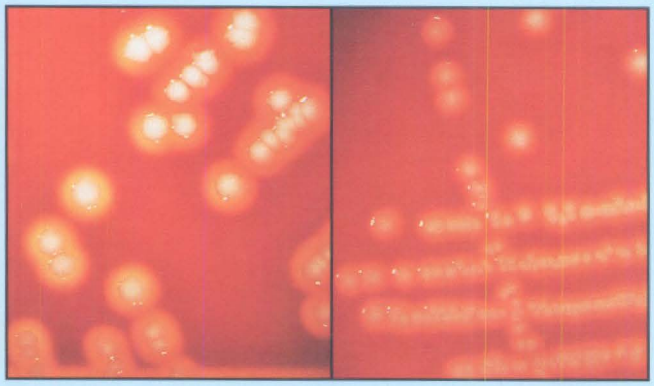
6 Kornacki, J.L. and Marth, E.H. (1982) *J. Food Prot.* **45,** 1051–1067.

Some Detection Methods for *E. Coli* toxins illustrated



α haemolysis. EntHly haemolysis.

E. coli α and EntHly haemolysis

Note the greater clarity and more sharply defined edges of the α-haemolytic zones which are usually visible after 3–4 hours incubation.

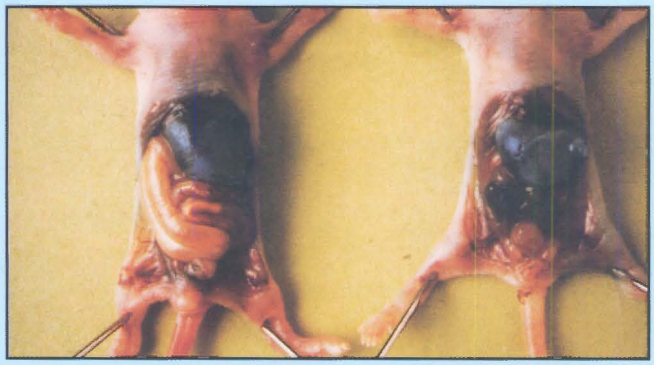
EntHly haemolysis develops after overnight incubation and only on sheep blood agar made with washed cells. Presence of haemolysis on both washed and unwashed blood agar plates is attributable to α (or β) haemolysins.

Reference:
Beutin, L., Prada, S., Zimmerinss, S., Stephan, R., Orskov, I. and Orskov, F. (1988). *Zentralbil. Bakteriol. Hyg. A.* **267,** 576–588.

Photographs supplied by Dr Barbara Gerten, Oxoid GmbH, Wesel.

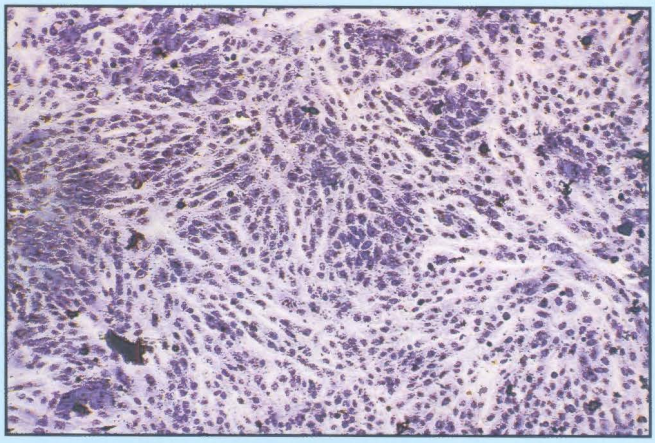
The rabbit ileal loop test for *E. coli* heat-labile toxin. The distended fluid-filled segment indicates the presence of toxin. A negative test is indicated by the lack of fluid in the segment below.

Photograph supplied by Dr Henry Smith, Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, UK.

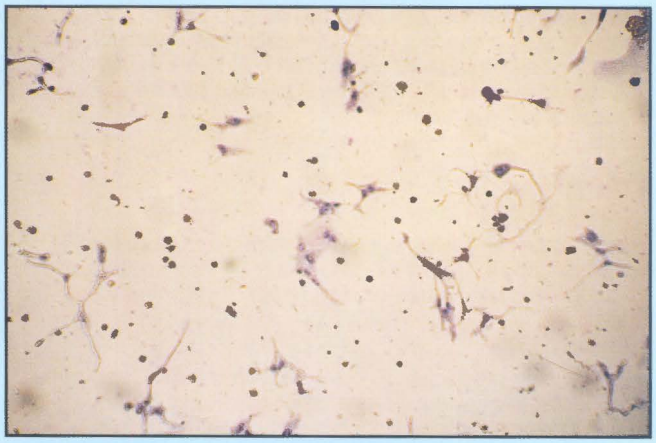


The infant mouse test for *E. coli* heat-stable toxin. The distended fluid-filled intestine of the mouse on the left indicates the presence of toxin. The mouse on the right shows no effect.

Photograph supplied by Dr Henry Smith, Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, UK.

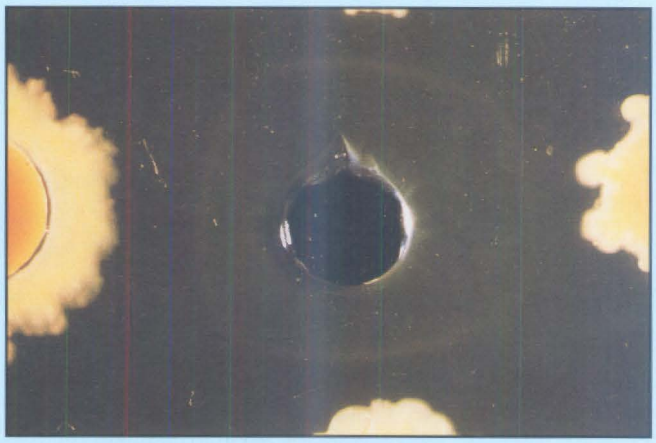


Normal Vero cells in culture. Giemsa stain.



Appearance of Vero cells following exposure to verocytotoxin produced by *E. coli.* Giemsa stain.

Photographs supplied by Dr Henry Smith, Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, UK.



The Biken gel-diffusion test for *E. coli* heat-labile toxin. The precipitin line in the gel is formed where toxin diffusing from the *E. coli* strains binds with antibodies to anti-heat-labile toxin diffusing from the central well.

Photograph supplied by Dr Henry Smith, Laboratory of Enteric Pathogens, Central Public Health Laboratory, Colindale, UK.

E. coli Toxin Detection Kits

The kits are simple to operate but are reliable for toxin detection in the majority of laboratories where sophisticated equipment or special skills in chemistry are not available.

Both industrial and clinical applications benefit from their use. Improved quality control of foods and raw materials, better detection of food poisoning outbreaks and rapid clinical diagnosis are examples of these benefits. The ability to carry out such tests in the laboratory, without sending them to external reference laboratories, is an additional benefit.

RPLA Toxin Detection by RPLA

E. coli enterotoxin and verocytotoxin can be detected by performing a simple dilution assay, using reverse passive latex agglutination (RPLA).

Principles of the Assay

Polymer latex particles are sensitised with purified rabbit anti-serum which is reactive either with *E. coli* LT or verocytotoxin. The latex particles will agglutinate in the presence of the toxins. Agglutination results in the formation of a lattice structure. On settling, this forms a diffuse layer on the base of a V-bottom microtitre well. If *E. coli* toxin is absent, or at a concentration below the assay detection level, no such lattice structure can be formed, and a tight button will therefore be observed. The use of polymyxin B solution facilitates the release of verocytotoxins.

VTEC RPLA
Code: TD960

A reverse passive latex agglutination test for the detection of verocytotoxins VT1 and VT2 produced by Escherichia coli cultured from food and faecal samples.

Introduction

Verocytotoxin-producing *E. coli* (VTEC) are transmitted through food, water and person-to-person contact, and are known to cause illnesses ranging from self-limiting watery diarrhoea to haemorrhagic colitis, Haemolytic Uraemic Syndrome (HUS) and thrombotic thrombocytopaenic purpura. These illnesses can be fatal, making the increasing incidence of VTEC contamination a cause for widespread concern.

Unlike other tests which detect the presence of strains such as *E. coli* 0157 (of which some, but not all, produce verocytotoxins), the Oxoid VTEC-RPLA test detects the toxins themselves, providing a clear and specific indication of VT1 or VT2 production. This overcomes the problem of positive results from other latex and culture assays (i.e. those which detect the organisms rather than the toxin) where non-toxin-producing *E. coli* 0157 strains are present. Similarly, it also overcomes the problem of negative results in cases where non-0157 strains are responsible for toxin production.

The test can be used with isolates cultured from both food and faecal samples.

VET-RPLA
Code: TD920

A test for the detection of Vibrio cholerae enterotoxin and Escherichia coli heat-labile (LT) enterotoxin in culture filtrates.

Enterotoxigenic *E. coli* (ETEC) strains are a common cause of diarrhoea in developing countries and in travellers moving between countries. The LT toxin can easily be detected in culture filtrates of the suspected strains. *V. cholerae* enterotoxin (CT) is antigenically similar to LT toxin and can be detected more easily because of the higher level of toxin produced in culture filtrates.

Toxin Detection by Enzyme Immunoassay

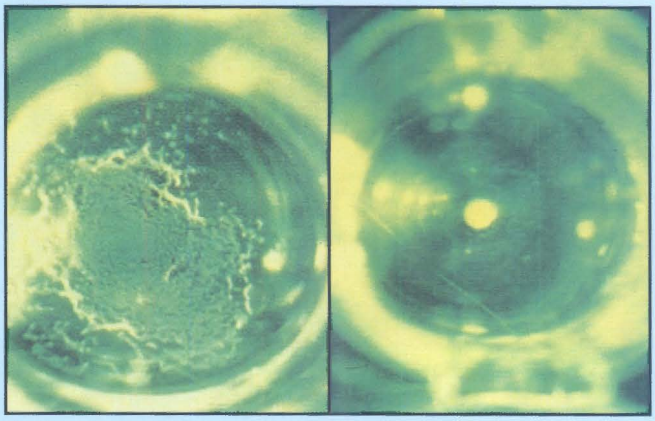
E. coli ST EIA
Code: TD700

A kit for the detection of E. coli heat-stable (ST) enterotoxin in culture filtrates or supernatants by enzyme immunoassay (EIA).

Enterotoxigenic *E. coli* (ETEC) are a common cause of infantile diarrhoea in developing countries and a cause of Traveller's Diarrhoea. These ETEC strains may produce heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST) or both. This kit together with VET-RPLA (code TD920) will determine the toxin production of any suspected ETEC strain.

The EIA test may be carried out on culture filtrates or supernatants. Performed in microtitre wells, the test is a competitive EIA with solid-phase toxin coated on the wells. The toxin used is a pure synthetic *E. coli* heat-stable preparation. The antibody-enzyme conjugate is composed of a monoclonal antibody linked to horseradish peroxidase. This conjugate will bind to the solid-phase toxin or to the toxin in the culture fluid. The presence of toxin in the culture fluid will compete with the solid-phase toxin and thus reduce the solid-phase binding of conjugate. After washing the well, such competition will result in a reduced colour intensity on addition of the substrate, which is clearly distinguished from a coloured negative result.

Full instructions for using the *E. coli* toxin detection kits are supplied with each kit.



Positive Negative

Testing for *E. coli* heat-labile (LT) toxin using the Oxoid VET-RPLA kit (Reversed passive latex agglutination).

ROW A Negative control
B Positive control
C Column 1 culture medium control
D Positive tests
E Column 1 positive Column 2 negative
F Column 1 negative Column 2 positive
G Negative
H Column 1 negative Column 2 positive

Testing for *E. coli* heat-stable (ST) toxin using the Oxoid *E. coli* ST toxin enzyme immunoassay (EIA) kit.

The Antigens of *E. coli* and Immunological Identification

Antigenically *E. coli* is very diverse and consequently a great number of serotypes have been recognised. This is in marked contrast to the genus Shigella in which antigenic diversity is not great.

E. coli possesses cell (somatic), flagellar and capsular antigens. Somatic antigens are named O antigens. O antigens are the polysaccharide portion of lipopolysaccharide attached to the cell membrane. Flagellar antigens are termed H antigens after "hauch", the German for breath, because of the very fine condensed-breath-like appearance of motile growth on solid culture medium. H antigens are absent from non-motile strains. K antigens are present on capsulated strains.

All three types of antigen are used in typing isolates following a scheme introduced by Kauffmann.[1]

E. coli strains are divided into serogroups based on O antigens and further differentiated into serotypes based on the H antigens. K antigens are markers of virulence, certain K antigens being present on strains associated with particular disease conditions. Serogroup and serotype identification is based on agglutination reactions using antisera prepared against O and H antigens. Numbers are given to O and H antigens and strains that react against the corresponding antibodies are identified by the numbers. The absence of H antigen on non-motile strains is noted e.g. 0157:H7 (H antigen present) or 0157:H-(H antigen absent). Although it is desirable that the presence of the H7 antigen should be determined in serogroup 0157, it is not essential as both motile and non-motile strains cause enterohaemorrhagic disease and isolation of any 0157 strain must be regarded as significant.

E. coli serological classification does not follow the convention of Salmonella classification that gives species names to strains possessing particular combinations of O and H antigens.

Groups of *E. coli* possessing particular O and H antigens tend to be associated with the different virotypes (see Table 2). *E. coli* 0157:H7 is included with other verocytotoxigenic serotypes in the enterohaemorrhagic *E. coli* (EHEC) group.

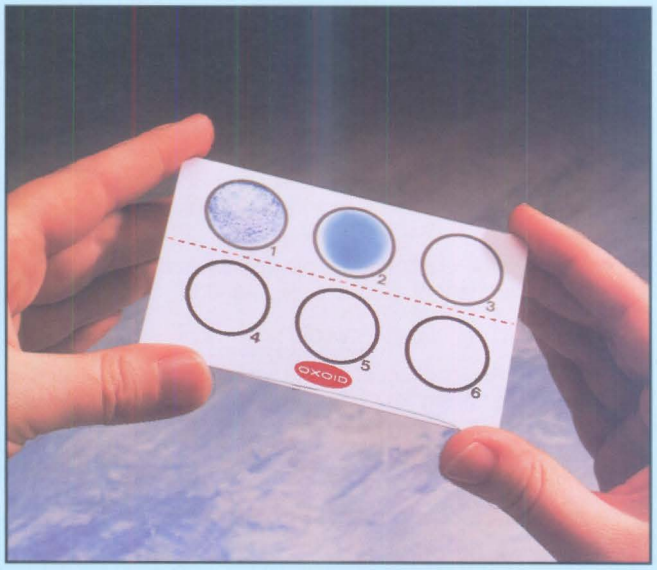
Classically, serological identification is carried out using polyclonal antibodies prepared by immunising rabbits with microbial cells of known antigenic type. False identification arising from cross-reacting antibodies to antigens shared with other genera or species is overcome by treating the immune sera with cells possessing the corresponding shared antigen. The cells with unwanted antibody bound to them are then removed from the immune serum leaving it with enhanced specificity. Using this procedure, false positive reactions when testing strains that may share antigens with different species are largely eliminated, e.g. when testing for *E. coli* 0157 which may share the 0157 antigen with *Escherichia hermanii*.

Serological reactions may be visually enhanced if the antigen is bound to an inert carrier, e.g. latex. The ability to colour latex particles makes agglutination reactions even easier to see.

Agglutination tests for most serotypes of *E. coli* are performed, without the benefit of latex enhancement, on glass slides or in glass tubes. The Oxoid 0157 Latex Agglutination kit is described on page 48.

Reference

1 Kauffmann, F. (1947) *J. Immunol.* **57,** 71–100.



Agglutination of blue latex particles in the Oxoid *Escherichia coli* 0157 Latex test which identifies as *E. coli* 0157 this non-fermenting strain of *E. coli* taken from a culture on sorbitol-MacConkey agar. The other test shows a negative reaction.

Oxoid Marketing Collection.

Shigella

Differentiation of *Shigella species* and *E. coli* can be difficult because they are so similar. The major phenotypic differences are shown in Table 5 but there are strains of *E. coli* and Shigella and species of Shigella that show exceptions to these general rules. Probably the best way is to increase considerably the number of biochemical tests done on an isolate. The pattern of results should be compared with both *E. coli* and the different species of Shigella.

Shigella are separated into four groups A, B, C, and D which correspond to the four species. Details are given in Table 6.

Shigella and enteroinvasive strains of *E. coli* are particularly close phenotypically and their similarity is increased by a close antigenic relationship. Serologically, *S. dysenteriae* 3 and *E. coli* 0124, *S. boydii* 8 and *E. coli* 0143 and *S. dysenteriae* 12 and *E. coli* 0152 appear equivalent. Indeed, it seems likely that some cases diagnosed as shigellosis are actually *E. coli* infections.

The following generalisations are a useful guide:

1 Shigella cultures are always non-motile (see illustration, page 56) and lysine is not decarboxylated.

2. Gas production by shigellae is rare although it may be seen occasionally with strains of *Sh. flexneri*, *Sh. boydii* and *Sh. dysenteriae*.

3 Cultures that ferment mucate, utilise citrate or produce alkali on acetate agar are likely to be *E. coli*.

4 Cultures that decarboxylate ornithine are most likely to be *Sh. sonnei* and less likely to be *E. coli*.

5 Cultures that ferment sucrose are likely to be *E. coli*.

6 Approximately one-third of *E. coli* strains will ferment salicin but shigellae do not.

Detailed information about identification of Shigellae is available in textbooks and Cowan and Steel's *Manual for the Identification of Medical Bacteria*.[1]

Identity should be confirmed by serological testing using antisera to members of groups A, B, C and D.

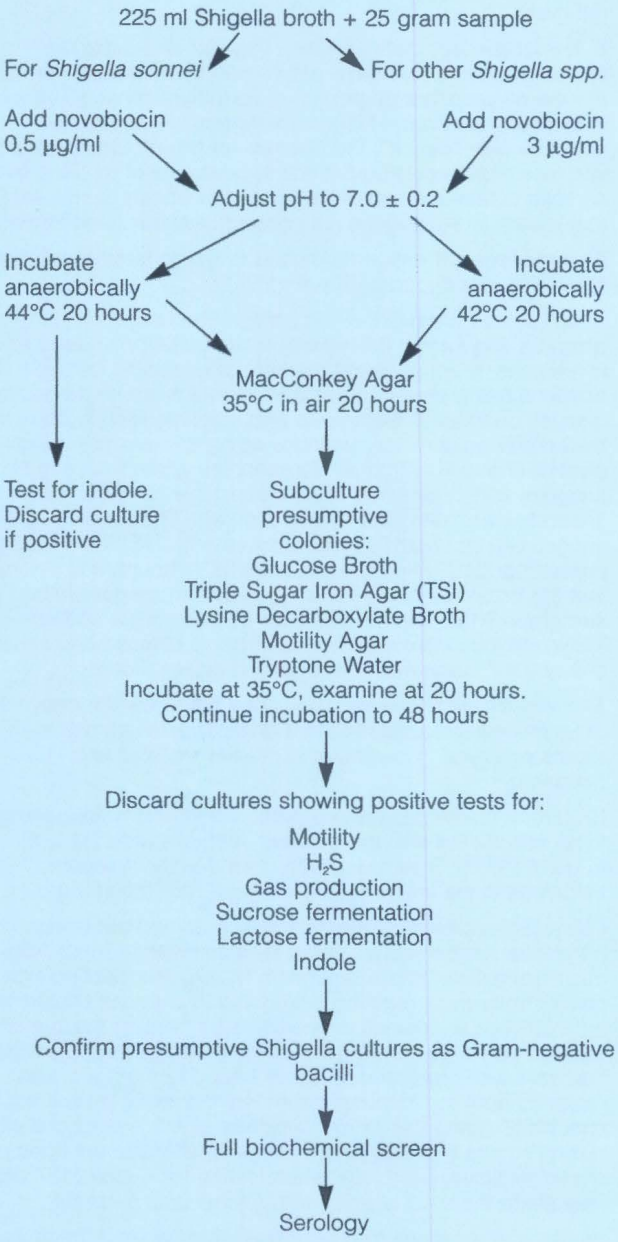
Reference

1 Cowan and Steel's *Manual for the Identification of Medical Bacteria*, 3rd edition 1993. Barrow, G.I. and Feltham, R.K.A. (eds). Cambridge University Press.

Table 5 – Major Phenotypic Differences Between *E. coli* and Shigellae

	E. coli	Shigella
Motility	+	–
Lactose fermentation	+	–
Indole production	+	–
Gas from glucose	+	–

FDA-BAM Method for Enrichment Culture of Shigella Species in Foods



This is an abbreviated version of the procedures used. Microbiologists intending to follow this method should consult the FDA Bacteriological Analytical Manual for full details.

Reference

FDA Bacteriological Analytical Manual, 8th edition 1995, Chapter 6. AOAC International, Arlington Va.

Liquid Media for *Shigella species*

The shigellae are not very demanding nutritionally and will grow satisfactorily in many non-selective nutrient media although growth may not be as abundant as that of the escherichiae. Problems arise in selective enrichment culture because the close similarities between Shigella and *E. coli* make it difficult to suppress *E. coli* without also severely inhibiting *Shigella species*. 1% peptone water has been used for enrichment culture of shigellae and addition of novobiocin has been reported to improve the isolation rate of *Sh. sonnei* and *Sh. flexneri* from various foods.[1] However, it is preferable to employ phosphate buffered peptone water to minimise the damaging effects of pH fall during growth.

Some success has been achieved using Hajna's.[2] GN (Gram-negative) Broth. GN Broth is a medium formulated for the cultivation of Gram-negative organisms from all types of specimens. Unfortunately many genera of Enterobacteriaceae will grow in this medium and although it favours the growth of *Shigella spp.* it is not selective for them. GN Broth is inhibitory to Gram-positive organisms because of the sodium citrate and sodium desoxycholate it contains. These to some extent also inhibit coliforms. A greater than usual content of mannitol is present, intended to accelerate the growth of the *Shigella spp.* capable of fermenting this carbohydrate. The performance of GN Broth is improved if the pH value is adjusted to 6.0–7.0 after the addition of the food sample.[3] The isolation rate may be increased if Selenite Cystine Broth is used in conjunction with GN Broth. Enrichment in Selenite F Broth may result in an increased isolation rate of shigellas but performance of the medium is inconsistent.

Beckers and Soentoro[4] also used GN Broth in a method they devised to improve detection of shigellae in foods. *Sh. flexneri* and *Sh. boydii* were detected in samples of shrimp contaminated with 1 cell in 25 gram and in a variety of vegetables contaminated with 25 cells in 25 gram. The procedure was unsuccessful when used with samples of minced beef even though contaminated with many more Shigella cells.

GN Broth modified by the addition of DL serine is specified in a procedure for the isolation of shigellae from water.[5]

Culture of shigellae, like that of other organisms, is made more difficult if the cells have been damaged during food processing and preservation. In a study of the capability of selective media to detect heat-injured *Shigella flexneri*[6] only Shigella Broth[7] permitted complete repair of injured cells. This medium is a phosphate-buffered tryptone glucose broth containing Tween 80. It does not contain bile salts and novobiocin is used as a selective agent. Addition of pyruvate improved the performance of all the other media tested.

Whilst it is likely that the usual three-stage procedure of resuscitation, selective enrichment and selective plating is needed to detect Shigella in foods by culture techniques, there is no agreement about choice of media and methods and much work remains to be done to optimise isolation procedures. A procedure found to be satisfactory by AOAC is described in the FDA Bacteriological Analytical Manual,[7] and is summarised in tabular form on page 54. In this procedure cultures are incubated under anaerobic conditions to take advantage of the ability of *Shigella spp.* to compete effectively against coliforms in a low carbohydrate Shigella Broth.

Shigella sonnei is able to grow at 44°C and is relatively tolerant at this elevated temperature towards novobiocin contained in the medium.[8] Anaerobic incubation may also improve performance by giving protection from intracellular production of toxic oxygen products formed under aerobic conditions. In earlier studies Fishbein, Mehlman and Wentz[9] had investigated adverse competitive ratios of minimal numbers of Shigella in the presence of high numbers of competitors obtained from ground meat and crab meat in a most probable number technique. The medium used was Tryptone Soya Broth containing 10 µg/ml of tetracycline. A method specified by the American Public Health Association[10] for the detection of Shigella in water recommends enrichment in GN Broth for 18 hours at 35–37°C. Formulae for GN and Shigella Broths are given on page 56.

References

1 Rutsch, C. (1987) Inaugural Dissertation, Freie Universität Berlin, Berlin. Journal number 1312, 116–120.
2 Hajna, A.A. (1955) *Publ. Hlth Lab.* **13,** 83–89.
3 Morris, G.K. (1984) Shigella. In: *Compendium of Methods for the Microbiological Examination of Foods*, 2nd edition. American Public Health Association, Washington DC.
4 Beckers, H.J. and Soentoro, P.S.S. (1989) *Zbl. Bakt. Hyg.* B187, 261–265.
5 The Microbiology of Water (1994) Part 1 – Drinking Water. Report on Public Health and Medical Subjects number 71. *Methods for the Examination of Water and Associated Materials*, London, HMSO.
6 Smith, J.L. and Dell, B.J. (1990) *J. Food Prot.* **53,** 141–144.
7 AOAC Bacteriological Analytical Manual (1995) 8th edition, Appendix 3.49, M136. AOAC International, Arlington Va.
8 Mehlman, I.J. (1984) *FDA Bacteriological Manual*, 6th edition, Chapter 9. AOAC International, Arlington Va.
9 Fishbein, M., Mehlman, I. and Wentz, B. (1971) *J. Assoc. Off. Anal. Chem.* **54,** 109–111.
10 Geldreich, E.E. (1980) in *Standard Methods for the Examination of Water and Wastewater*. 15th edn. American Public Health Association. Washington DC. P.838.

GN Broth

GN Broth

Formula

	grams/litre
Tryptose	20.0
Glucose	1.0
D-Mannitol	2.0
Sodium citrate	5.0
Dipotassium hydrogen phosphate	4.0
Sodium desoxycholate	0.5
Potassium dihydrogen phosphate	1.5
Sodium chloride	5.0
Glucose	1.0

pH 7.0 ± 0.2

GN Broth was developed by Hajna.[1] It is an enriched medium originally devised for the detection of Gram-negative pathogens in faeces, sputum, blood and urine and on eating and drinking utensils. The medium is not selective for Shigella but its use is reported to increase the isolation rate of *Shigella spp.* GN Broth is specified for use in examining foods for the presence of Shigella.[2] Modified by the addition of serine, it is also specified in a procedure for the isolation of *Shigella spp.* from water.[3]

Beckers and Soentoro[4] supplemented GN Broth with novobiocin 10 mgm/litre in a method to detect *Shigella spp.* in foods.

Following incubation at 37°C, subculture was made on SS Agar with and without 7.5 mgm/litre of streptomycin.

The procedure was more satisfactory with shrimp and a variety of vegetables than with minced beef.

Tryptose is used as a nutrient because of its ability to produce rapid and profuse growth. The medium is buffered and sodium desoxycholate and sodium citrate are included to inhibit Gram-positive bacteria. Sodium citrate has an additional function of serving as an anticoagulant in blood culture. The small amount of glucose present was calculated to approximate to that usually found in infusion broths. Mannitol is present in greater amount than is usual; the high proportion of mannitol to glucose in the fermentable carbohydrates present is intended to limit the growth of non-mannitol-fermenting *Proteus spp.* and stimulate growth of Shigella and Salmonella organisms capable of fermenting mannitol.

Proteus and Pseudomonas will grow in the medium, but to a lesser extent in the first six hours of incubation.

GN medium should be subcultured for the first time at about 6 hours to maximise the isolation rate of Shigella and Salmonella. If desired, incubation can then be continued and further subcultures made at 24 hours.

References

1 Hajna, A.A. (1955) *Pub. Hlth* **13,** 83–89.
2 Morris, G.K. (1984) Shigella. In: *Compendium of Methods for the Microbiological Examination of Foods.* 2nd edition. APHA Washington DC.
3 The Microbiology of Water (1994) Part 1 – Drinking Water. Report on Public Health and Medical Subjects Number 71. *Methods for the Examination of Water and Associated Materials.* HMSO London.
4 Beckers, H.J. and Soentoro, P.S.S. (1989) *Zbl. Bakt. Hyg.* B **187,** 261–265.

Shigella Broth

Formula

	grams/litre
Tryptone	20.0
Dipotassium hydrogen phosphate	2.0
Potassium dihydrogen phosphate	2.0
Sodium chloride	5.0
Glucose	1.0
Tween 80	1.5 ml
Water	1000 ml

pH 7.0±0.2

Antibiotic supplement: Novobiocin	50 mg distilled water 1000 ml

Sterilise by filtration.

Reference

AOAC Bacteriological Analytical Manual (1995) 8th edition. Appendix 3.49 M136. AOAC International, Arlington Va.

Electron micrograph of two Shigella cells. Note the complete absence of flagellae.

Photograph: Dr Peter Hawtin, Public Health Laboratory, Southampton, UK.

Selenite Broth

Selenite Broth
Code: CM395

An enrichment medium for the isolation of Salmonella from faeces and food products. Selenite Broth may also improve the isolation rate for Shigella sonnei.

Formula

	grams/litre
Peptone	5.0
Lactose	4.0
Sodium phosphate	10.0
pH 7.1 ± 0.2	

Directions

Dissolve 4 grams of sodium biselenite L121 in 1 litre of distilled water and then add 19 grams of CM395.

Warm to dissolve, mix well and fill out into containers to a depth of 5 cm. Sterilise in a boiling water bath, or in free flowing steam, for 10 minutes. **Do not autoclave**.

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added as a solution to this medium.

Robertson[1] reported miscarriages and possible teratogenic effects on pregnant laboratory assistants which may have been caused by ingested sodium biselenite. Oxoid therefore removed this substance from the powdered medium.

Although no further reports have been received, sodium biselenite is now considered to be very toxic and should be handled with great care.

Absence of sodium biselenite from the basal medium also facilitates procedures in which the selective agent is added at some time after incubation commences in order to allow resuscitation to occur.

Sodium Biselenite (Sodium hydrogen Selenite)
Code: L121

Directions

Dissolve 4 g in 1 litre of distilled water and use this solution to reconstitute the base medium.

Toxic by inhalation and if swallowed. Danger of cumulative effects.

Description

Klett[2] first demonstrated the selectivity inhibitory effects of selenite and Guth[3] used it to isolate *Salmonella typhi.* It was twenty years later before Leifson[4] fully investigated selenite and promoted wide use of the medium.

Selenium toxicity to certain microorganisms is not fully understood but it is suggested that it reacts with sulphur and sulphadryl groups in critical cell components.[5,6]

Proteus and *Pseudomonas species* appear to be resistant to its effects.[5] Lactose is added as a fermentable carbohydrate to prevent a rise in pH value during incubation because any increase in pH will reduce the selective activity of selenite. The fact that *Proteus* and *Pseudomonas species* do not ferment lactose may explain why they escape inhibition.

There have been many modifications and alterations to the original medium described by Leifson, including substitution of mannitol to replace lactose (Mannitol Selenite Broth CM399), addition of cystine (Selenite Cystine Broth CM699), brilliant green, sodium taurocholate, sulphapyridine and streptomycin. The performance of these modifications has been investigated but with no overall agreement.[7]

Incorporation of Selenite Broth in isolation protocols for shigellae may improve detection of *Shigella sonnei* but results are inconsistent.

Technique

For routine purposes Selenite Broth cultures should be incubated at 35°C for 18 to 24 hours and then subcultured on any combination of greater and lesser inhibitory selective agar for Enterobacteriaceae. The development of *Escherichia coli* and *Proteus species* is not indefinitely retarded in selenite media. Where the initial proportion of these organisms is high it is often advantageous to subculture onto the solid media after 6 hours as well as after 18 hours.

If a high proportion of debris is present in the sample of material being examined, the selective powers of the selenite may be nullified. This is well established in the examination of faeces and egg powder. It is common practice to emulsify the specimen in sterile saline, allow the gross particles to settle, and inoculate the medium with the supernatant. An alternative method is as follows: add 2 to 3 grams of solid specimen to 15 ml of saline in a wide-necked 1 oz bottle, emulsify and separate the debris by slowly pressing a plug of cotton-wool down through the suspension. Withdraw approximately 1 ml of the supernatant and inoculate 10 ml of Selenite Broth.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label. Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Subculture to MacConkey Agar.

Precautions

Discard the prepared medium if large amounts of reduced selenite can be seen as a red precipitate in the bottom of the bottles. Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6–12 hours incubation.[8] Take subcultures of broth from the upper third of the broth column, which should be at least 5 cm in depth.

References

1 Robertson, D.S.F. (1970) *Lancet* **i,** 518–519.
2 Klett, A. (1900) *Zeitsch. für Hyg. und Infekt.* **33,** 137–160.
3 Guth, F. (1916) *Zbl. Bakt. I. Orig.* **77,** 487–496.
4 Leifson, E. (1936) *Amer. J. Hyg.* **24,** 423–432.
5 Weiss, K.F., Ayres, J.C. and Kraft, A.A. (1965) *J. Bact.* **90,** 857–862.
6 Rose, M.J., Enriki, N.K. and Alford, J.A. (1971) *J. Food Sci.* **36,** 590–593.
7 Fagerberg, D.J. and Avens, J.S. (1976) *J. Milk, Food Technol.* **39,** 628–646.
8 Chattopadhyay, W. and Pilford, J.N. (1976) *Med. Lab. Sci.* **33,** 191–194.

Selenite Cystine Broth

Selenite Cystine Broth
Code: CM699

An enrichment medium used for the isolation of Salmonella and shigellae from foods and faeces.

Formula

	grams/litre
Tryptone	5.0
Lactose	4.0
Disodium phosphate	10.0
L-Cystine	0.01
pH 7.0 ± 0.2	

Directions

Dissolve 4 g of Sodium Biselenite L121 in 1 litre of distilled water and then add 19 g of Selenite Cystine Broth Base CM699. Warm to dissolve and dispense into containers to a depth of at least 5 cm. Sterilise by placing in free flowing steam for 15 minutes. **Do not autoclave**.

To minimise any possible risk of teratogenicity to laboratory workers the sodium biselenite is not included in the dry powder but should be prepared separately as a solution to which the Selenite Cystine Broth Base is added.

Description

Selenite Cystine Broth Base CM699 is modified from the formula of Leifson[1] with added cystine.[2] This addition has given favourable results in many studies.[3]

The effect of the cystine may be due to its reducing abilities which will lower the toxicity of selenite to microorganisms and/or the extra organic sulphur provided may have a sparing effect on the critical sulphur components of the bacteria, again reducing the selective effect of the selenite.

Selenite Cystine Broth is used for enrichment culture of salmonellae from faeces, foodstuffs and other materials. The formulation corresponds to that recommended by the AOAC[4] for detection of Salmonella in foodstuffs, in particular egg products. It is included among the standard methods media for the American Public Health Association.[5,6] It also complies with the requirements of the United States Pharmacopoeia.[7] Use of Selenite Cystine Broth with GN Broth is recommended to improve the isolation rate of shigellae.[8]

Technique

The proportion of sample in the enrichment broth should not exceed 10–20% (1 or 2 grams in 10–15 ml). Solid material is added to the normal strength broth. Liquid samples are mixed with double strength medium in the ratio of 1 to 1. Incubate for 12–24 hours at 35–37°C.

Subculture to any combination of greater and lesser inhibitory, selective agars for the Enterobacteriaceae including MacConkey and XLD Agars.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028
Shigella flexneri ATCC® 12022
Negative control:
Escherichia coli ATCC® 25922
Subculture to MacConkey Agar

Precautions

Observe the precautionary comments made about sodium biselenite in Selenite Broth Base CM395. Discard the prepared medium if large amounts of reduced selenite can be seen as red precipitate in the bottom of the bottle.

Do not incubate longer than 24 hours because the inhibitory effect of selenite is reduced after 6–12 hours incubation.[9]

References

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7 United States Pharmacopoeia XXI (1980) Microbial Test Limits.
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Agar Media for *Shigella species*

Few agar media have been devised specifically for *Shigella species* and direct plating of food samples is unlikely to be successful. The close relatedness of shigellae to *E. coli* ensures that the susceptibility of shigellae to selective agents is very similar to that of *E. coli*. Consequently suppression of *E. coli* growth in mixed culture without at the same time inhibiting the growth of Shigella is very difficult to achieve and depends heavily on compromise and balancing the functions of the medium. The potential for selective activity of DCA and SS media to shift from satisfactory inhibition of *E. coli* and other enteric organisms while at the same time permitting satisfactory growth of *Shigella spp.* is ever-present and manufacturers of culture media put considerable effort into maintaining the correct balance.

Selection of shigellae has generally to be accomplished by emphasising biochemical differences between Shigella and the accompanying organisms on media with low or intermediate inhibitory activity. On MacConkey Agar for example, shigella isolates appear as non-lactose-fermenting colonies (although *Sh. sonnei* may show late fermentation if incubation is prolonged) and may readily be detected provided the accompanying flora is not excessive. Shigella colonies differ in appearance from other non-lactose-fermenting colonies and this is a useful guide to selecting colonies for confirmatory tests.

In situations where either Shigella or Salmonella may be present it is necessary to use media having intermediate selectivity capable of growing either genus. Media that fall into the category and depend on lactose fermentation for primary differentiation include desoxycholate-citrate media (DCA) and Salmonella-Shigella (SS) media. Variations on both formulae exist, differing somewhat in selectivity. The failure of Shigella and most Salmonella to ferment lactose immediately distinguishes both genera from *E. coli*. DCA and SS media contain components that enable Salmonella to be distinguished from Shigella by blackening of the colonies resulting from hydrogen sulphide production.

Desoxycholate-citrate-lactose-sucrose (DCLS) agar has sucrose added to the formula to reduce the number of non-pathogenic, non-lactose-fermenting colonies submitted to further testing.

Many other media designed for growth of coliforms may be used with varying success for detection of shigellae. Amongst these are eosin-methylene blue agar (EMB) and Tergitol-7 agar.

The different species of Shigella vary in their ability to grow on any particular medium. Added to this difficulty is the effect of damage to cells caused during food processing. In an investigation of the capability of media to grow heat-injured *Shigella flexneri*, Smith and Dell[1] concluded that an agar medium made from lauryl tryptose broth and EMB Agar were the least inhibitory to the repair of injured cells and SS Agar and desoxycholate-citrate agar were amongst the least satisfactory. Addition of pyruvate to all the media tested improved recovery but the authors concluded that most media used for the isolation of shigellae do not permit recovery of heat-injured cells unless special techniques are incorporated into the isolation procedures.

Most work on formulating media to improve the recovery of shigellae has been directed towards clinical microbiology use and consequently the resulting media may not be entirely satisfactory for investigating foods. Taylor[2] developed a family of 3-xylose-lysine media, each designed for a specific purpose. Of this family, Xylose-Lysine Desoxycholate (XLD) is the only one that has become widely accepted. XLD Agar was designed for isolation of *Shigella spp.* and other enteric pathogens in a clinical laboratory setting; the medium is now also used extensively in food microbiology but generally for isolation of Salmonella.

Shigellas are relatively inactive biochemically. XLD Agar was designed to exploit this characteristic and shigellae are identified by their negative characteristics. Xylose was chosen as a fermentable carbohydrate because it is fermented by all Enterobacteriaceae apart from *Shigella* and *Providencia spp.* Also the medium was made suitable for Salmonella detection by incorporating lysine. Decarboxylation of this amino-acid causes the pH of the medium to revert to alkaline following initial acidification caused by xylose fermentation, thus distinguishing Salmonella from non-pathogenic members of the Enterobacteriaceae.

In order to prevent any lysine-positive coliforms present causing the pH of the medium to rise, lactose and sucrose are included to produce copious quantities of acid. The reversion of pH to alkaline would produce Salmonella colonies similar in appearance to those of Shigella if a further identification feature for Salmonella were not present. This need is fulfilled by hydrogen sulphide production from components of the medium. As a result Salmonella colonies appear different from Shigella colonies because they have a black centre.

A less radical approach to formulating a medium directed towards *Shigella spp.* was taken by King and Metzger[3] working at the Hektoen Institute. In contrast to XLD Agar the formula follows established practice of including bile salts as an inhibitor but a greater quantity of peptone than is usual is included to protect against excessive inhibition by the bile. Lactose, salicin and sucrose are present to differentiate pathogenic from non-pathogenic Enterobacteriaceae. The pH indicator system consisting of a combination of bromothymol blue and Andrade's indicator is novel in its application to solid media and was chosen because of its lack of toxicity. In evaluation tests the originators found Hektoen Agar to perform significantly better than EMB and SS Agars for the isolation of *Shigella spp.*[4]

The superiority of XLD over MacConkey, DCA and SS Agars was confirmed by Sen and co-workers[5] for the recovery of *Sh. dysenteriae* type 1 and *Sh. flexneri* from faeces. Use of XLD medium alone or in combinations of media which include XLD resulted in significantly higher isolation rates than use of any of the other media alone or in combinations which excluded XLD.

In another study the performance of XLD Agar was compared against Hektoen Agar, SS Agar and EMB Agar for isolation from faecal specimens. Both XLD and Hektoen Agars were found to be better than SS and EMB Agars but XLD produced only half the number of false-positive cultures as Hektoen Agar.[6] Novobiocin may be added to Hektoen Agar to increase its selectivity, principally by inhibiting *Citrobacter* and *Proteus spp.*[7]

Shigella infections are most commonly waterborne; foodborne disease may arise from foods that have been contaminated with polluted water. Shigellae may be isolated from water by concentration on a filter membrane followed by enrichment in modified GN broth[8] and plating onto a selective agar. Hektoen and DCA are recommended[8] and in this procedure the selectivity of Hektoen Agar is enhanced by the addition of novobiocin. Desoxycholate-citrate Agar is modified by increasing the peptone content and the incorporation of DL serine. The inhibitory activity is enhanced by the addition of tetracycline.

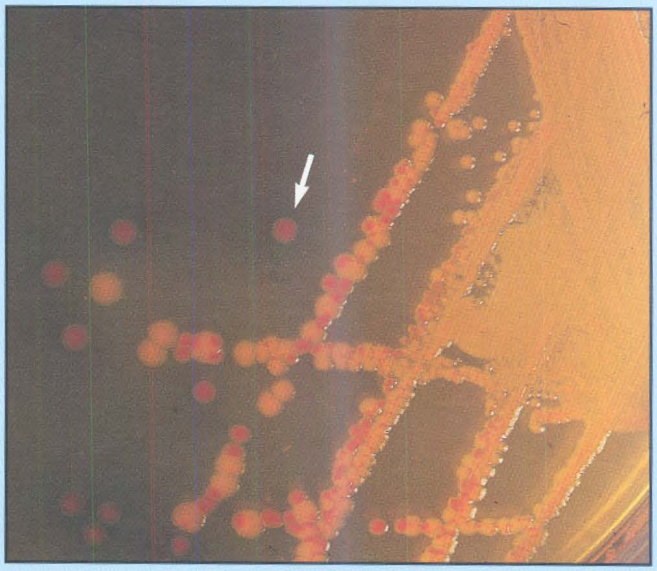
Media in the Oxoid range suitable for isolation of *Shigella spp.* are described on pages 56–58 and 61–69.

The formula of modifed DCA is given on page 68.

References

1 Smith, J.L. and Dell, B.J. (1990) *J. Food Prot.* **53,** 141–144.
2 Taylor, W.I. (1965) *Tech. Bull. Registry Med. Technol.* **35,** (9) reprinted in *Amer. J. Clin. Path.* (1965) **44,** 471–475.
3 King, S. and Metzger, W.I. (1968) *Appl. Microbiol.* **16,** 577–578.
4 King, S. and Metzger, W.I. (1968) *Appl. Microbiol.* **16,** 579–581.
5 Sen, D., Saha, M.R., Manna, B. and Pal, S.C. (1987) *J. Diarrhoeal Dis. Res.* **5,** 94–96.
6 Taylor, W.I. and Schelhart, D. (1971) *Appl. Microbiol.* **21,** 32–37.
7 Hoben, D.A., Ashton, D.H.A. and Petersen, A.C. (1973) *Appl. Microbial.* **21,** 126–129.
8 The Microbiology of Water (1994) part 1 – Drinking Water. Report on Public Health and Medical Subjects: Number 71. *Methods for the Examination of Water and Associated Materials.* HMSO, London.

Tergitol-7 Agar
Code: CM793
A selective medium which may be used to detect Shigella. A detailed description of this medium is given on page 33.



Appearance of *Shigella spp.* (arrowed) on Tergitol-7 Agar.

Desoxycholate Citrate Agar (Hynes)

Desoxycholate Citrate Agar (Hynes)
Code: CM227

A selective medium for the isolation of Salmonella and Shigella organisms.

Formula

	grams/litre
"Lab-Lemco" powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	8.5
Sodium thiosulphate	5.4
Ferric ammonium citrate	1.0
Sodium desoxycholate	5.0
Neutral red	0.02
Agar	12.0

pH 7.3 ± 0.2

Directions

Suspend 52 g in 1 litre of distilled water. Bring to the boil over gauze and flame, to dissolve completely.

Agitate to prevent charring. Dry the agar surface before use.

This medium is heat sensitive: avoid excessive or prolonged heating during reconstitution. Do not autoclave or remelt.

Description

An improved medium, based on the Hynes[1] modification of Leifson medium for the isolation of salmonellae and shigellae.

The improvement gives larger and more numerous colonies of *Shigella species* which can easily be picked off and emulsified in saline for slide agglutination tests.

Desoxycholate Citrate Agar (Hynes) is more selective than CM35. In particular, CM227 is more inhibitory to coliforms and *Proteus species*.

Technique

Inoculate the medium heavily with faeces or rectal swabs, spreading part of the original inoculum in order to obtain well separated colonies on some portion of the plate. Incubate for 18–24 hours at 35°C. If organisms are late developers or if no non-lactose fermenters are observed, incubate for a further 24 hours.

Colonies may be picked directly off the medium for serological and biochemical tests. It should be noted that *Escherichia coli* survives on the medium even though it does not usually grow – therefore colonial purity should be established by subculture onto a differential but less inhibitory medium, e.g. MacConkey Agar CM7.

Colonial Characteristics

(Following incubation at 35°C.)

The medium is clear and pale pink. Lactose-fermenting organisms produce pink colonies and may be surrounded by a zone of precipitated desoxycholic acid, which is due to acid production. The colonies of non-lactose fermenters are colourless, and due to their alkaline reaction they are surrounded by a clear orange-yellow zone of medium.

Escherichia coli – most strains are inhibited, but the few strains which grow produce pink umbilicated colonies 1–2 mm in diameter which may be surrounded by a zone of precipitation. *Aerogenes* colonies are domed and mucoid.

Shigella sonnei – the colonies grow from 1 mm diameter after 18 hours incubation to 2 mm after 38 hours; they are smooth and initially colourless, becoming pale pink on further incubation due to late lactose fermentation.

Shigella flexneri – colonies are colourless and similar in appearance to those of *Shig. sonnei*, but often with a narrow plane periphery around a central dome.

Salmonella paratyphi B – from 1 mm diameter after 18 hours incubation to 2–4 mm on the second day, when they are slightly opaque, dome-shaped, with a central black dot.

Salmonella typhosa – 0.25 to 1 mm in diameter after 18 hours and pale pink, a day later they are flat, conical, 2 mm in diameter, colourless and slightly opaque, often with a central grey dot.

Other Salmonella colonies – similar to those of *Salm. paratyphi B*. Non-pathogenic non-lactose fermenters, such as *Proteus* and *Pseudomonas species*, grow on the medium and may produce colonies which closely simulate those of the salmonellae or shigellae. *Proteus* colonies are often glossy (more translucent than those of the pathogens), with a large central black dot and a "fishy" odour.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control

Positive controls:
Salmonella typhimurium ATCC® 14028
Shigella sonnei ATCC® 25931

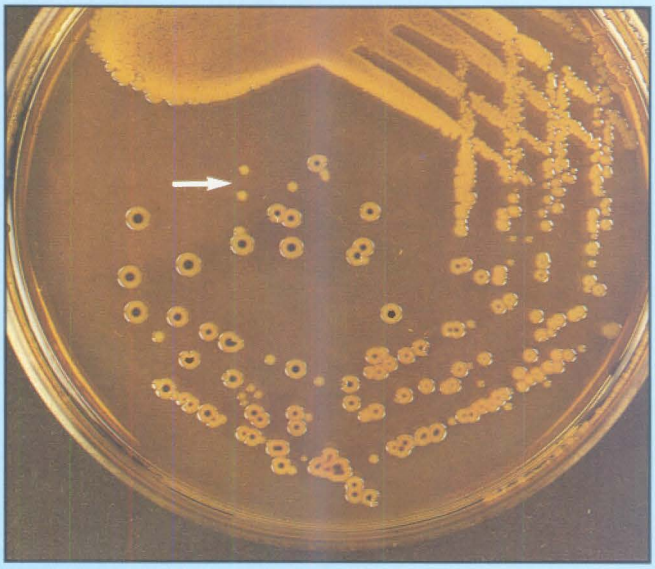
Negative control:
Enterococcus faecalis ATCC® 29212

Precautions

Note the precautions listed under Desoxycholate Citrate Agar CM35.

References

1 Hynes, M. (1942) *J. Path. Bact.* **54,** 193–207.



Appearance of *Shigella spp.* (arrowed) on Desoxycholate-citrate agar (Hynes).

Oxoid Marketing Collection.

Desoxycholate Citrate Agar (Leifson)

Desoxycholate Citrate Agar (Leifson)
Code: CM35

A modification of Leifson's medium for the isolation of intestinal pathogens.

Formula

	grams/litre
"Lab-Lemco" powder	5.0
Peptone	5.0
Lactose	10.0
Sodium citrate	5.0
Sodium thiosulphate	5.0
Ferric citrate	1.0
Sodium desoxycholate	2.5
Neutral red	0.025
Agar	15.0
pH 7.0 ± 0.2	

Directions

Suspend 48.5 g in 1 litre of distilled water. With frequent agitation bring to the boil over a gauze and flame to dissolve completely. Mix well and pour plates immediately. Dry the agar surface before use.

This medium is heat sensitive. Avoid excessive or prolonged heating during reconstitution. Do not autoclave, or remelt.

Description

An Oxoid modification of Leifson medium,[1] for the isolation and maximum recovery of intestinal pathogens. It is less selective and inhibiting than Desoxycholate Citrate Agar (Hynes) but colonial characteristics are identical on the two media.

See Desoxycholate Citrate Agar (Hynes) CM227 (page 61) for the description of colonies but note that DCA CM35 provides an opaque background against which one may more easily discern the clearing produced by alkali-producing pathogens.

The use of a less selective medium for direct sampling of faeces and a more selective medium for post-enrichment sampling, would be advantageous. Similarly, the less inhibitory medium is often preferable when shigellae are being sought as well as salmonellae.[2]

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control

Positive controls:
Salmonella typhimurium ATCC® 14028
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

Precautions

Observe the precautions about overheating shown under Directions.

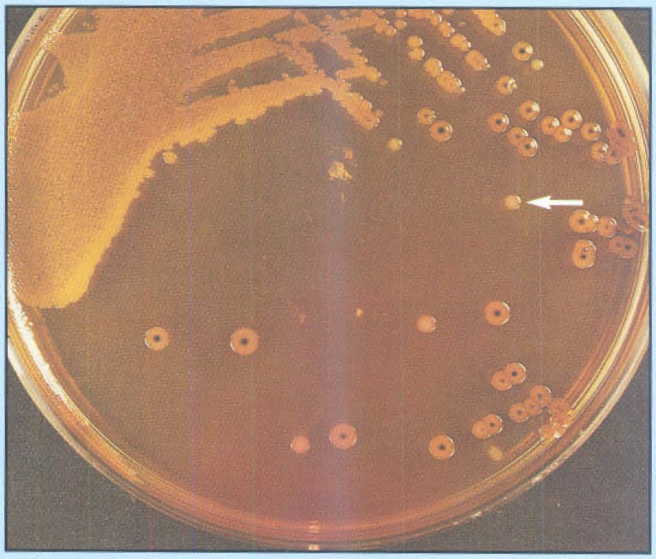
The medium is best used freshly prepared.

Stock cultures of *Shigella species* may become predominantly in the R-phase when subcultured away from DCA media. Such cultures are difficult to use for control purposes without first heavily streaking the cultures on DCA plates and picking off the few S-phase colonies, i.e. the macro-colonies, on the agar surface, for further subculture.

When making biochemical tests on colonies picked from the surface of DCA plates, purity subcultures should be carried out because the colony may be contaminated with *Escherichia coli* present as micro-colonies.

References

1 Leifson, E. (1935) *J. Path. Bact.* **40,** 581–599.
2 Fricker, C.R. (1987) *J. Appl. Bact.* **63,** 99–116.



Appearance of *Shigella spp.* (arrowed) on Desoxycholate-citrate agar (Leifson).

Oxoid Marketing Collection.

DCLS Agar

DCLS Agar
Code: CM393

A modified DCA containing sucrose to improve the accuracy of recognition of pathogenic Enterobacteriaceae.

Formula

	grams/litre
Special peptone	10.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Lactose	5.0
Sucrose	5.0
Sodium desoxycholate	2.5
Neutral red	0.03
Agar	12.0

pH 7.2 ± 0.2

Directions

Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Cool to 50°C and pour plates. **Do not autoclave.**

Description

DCLS Agar is a modified form of Desoxycholate Citrate Agar[1] which includes sucrose in its formulation. The addition of this fermentable carbohydrate increases the usefulness of the medium because non-pathogenic sucrose-fermenting organisms may be recognised by their red colonies, e.g. some *Proteus*, *Enterobacter* and *Klebsiella species*.

DCLS Agar reduces the number of false-positive subcultures when picking colonies and therefore improves the efficiency of isolation.

The special peptone used in DCLS Agar includes the nucleic acid factors, vitamins and carbon compounds of meat extract, as well as a rich variety of polypeptides. It has improved the growth of shigellae and salmonellae, but it should be noted that *Sh. sonnei* may exhibit a translucent, pink colony which should not be confused with the red *Esch. coli* colony.

The selectivity of DCLS Agar is similar to Desoxycholate Citrate Agar and it will grow *Vibrio species*, as well as salmonellae and shigellae, whilst inhibiting the growth of *Esch. coli*.

DCLS Agar may be inoculated directly from the specimen, or inoculated after enrichment for Salmonella through Selenite Broth CM395 and L121, Muller-Kauffmann Tetrathionate Broth CM343 or Tetrathionate Broth CM29. Media containing tetrathionate are not suitable for Shigella. The plates should be incubated overnight (18–24 hours) at 35°C and examined for the presence of pale, translucent or colourless colonies. Subcultures can be made into confirmatory media such as Kliger Iron Agar CM33 or Triple Sugar Iron Agar CM277 or picked for transfer to nutrient broth for subsequent motility tests and serological agglutinations.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared agar plates at 2–8°C.

Quality Control

Positive control:
Lactose/sucrose fermenters
Proteus vulgaris ATCC® 13315
Non-lactose/sucrose fermenters
Salmonella typhimurium ATCC® 14028
Sh. dysenteriae NCTC 9760

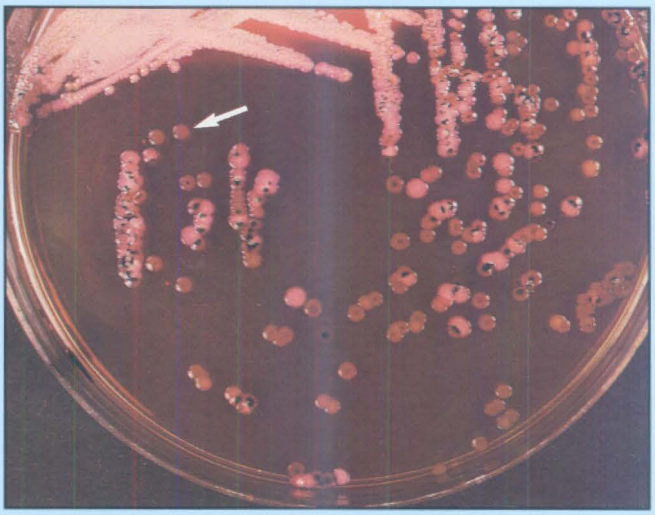
Negative control:
Staphylococcus aureus ATCC® 25923

Precautions

Boil the medium for the minimal period of time to get the agar into solution. Overheating reduces the agar gel strength and increases the degree of inhibition. It is therefore important not to hold the molten medium at 50°C for more than the short time required to distribute it into dishes.

Reference

1 Leifson, E. (1935) *J. Path. Bact.* **40,** 581–599.



Appearance of *Shigella spp.* (arrowed) on Desoxycholate Citrate-Lactose-Sucrose Agar (DCLS).

Oxoid Marketing Collection.

Hektoen Enteric Agar

Hektoen Enteric Agar
Code: CM419

A differential, selective medium for the isolation of Shigella and Salmonella species from enteric pathological specimens and from foods.

Formula

	grams/litre
Proteose peptone	12.0
Yeast extract	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile salts No. 3	9.0
Sodium chloride	5.0
Sodium thiosulphate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromothymol blue	0.065
Agar	14.0

pH 7.5 ± 0.2

Directions

Suspend 76 g of the medium in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. **Do not autoclave.** Cool to 50°C and pour plates.

Description

Hektoen Enteric Agar was developed by King and Metzger.[1] The high peptone content offsets the inhibitory effect of bile salts on *Shigella species* in particular. The additional carbohydrates (sucrose and salicin) give better differentiation than lactose alone and the lower toxicity of the double indicator improves recovery. The increased lactose content helps early recognition of slow lactose-fermenting organisms. The thiosulphate and ferric citrate are present to detect H_2S-producing organisms.

Taylor and Schelhaut[2] found the medium to be of value in the differentiation of pathogenic organisms and for better growth of shigellae.

Hoben *et al.*[3] added novobiocin 15 mg/litre to improve the selectivity of the medium by inhibiting *Citrobacter* and *Proteus species*. Rutsch[4] found that addition of novobiocin to Hektoen Agar improved isolation of *Sh. flexneri* and *Sh. sonnei* from a variety of foods.

Hektoen Enteric Agar meets the requirements of the APHA.[5]

Technique

Inoculate the medium with fresh faeces suspended in Ringers solution or inoculate directly with rectal swabs. Spread the inoculum to obtain well separated colonies. Incubate for 18–24 hours at 37°C. Further incubation will improve differentiation between shigellae and salmonellae.

Organism Characteristics

Shigella	Green, moist raised colonies.
Salmonella	Blue-green colonies with or without black centres.
Coliforms (rapid lactose/sucrose/ salicin fermenters)	Salmon-pink to orange colonies surrounded by a zone of bile precipitation.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared plates at 2–8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028
Shigella flexneri ATCC® 12022
Sh. sonnei ATCC® 25931
Sh. boydii NCTC 11462

Negative control:
Escherichia coli ATCC® 25922
Enterococcus faecalis ATCC® 29212

Precautions

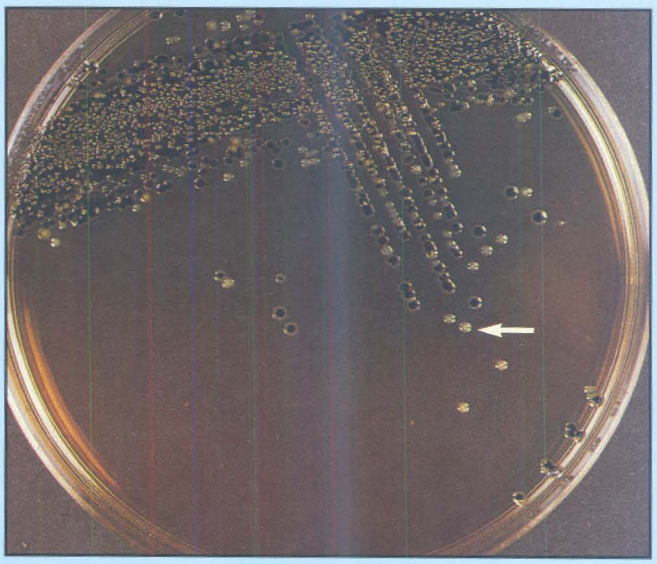
Do not overheat the medium or hold it molten for long periods.

Proteus species may resemble salmonellae or shigellae.

Further testing must be carried out to confirm the presumptive identification of organisms isolated on this medium

References

1 King, S. and Metzger, W.I. (1968) *Appl. Microbiol.* **16,** 577–581.
2 Taylor, W.I. and Schelhaut, D. (1971) *Appl. Microbiol.* **23,** 32–37.
3 Hoben, D.A., Ashton, D.H.A. and Peterson, A.C. (1973) *Appl. Microbiol.* **21,** 126–129.
4 Rutsch, C. (1987) Inaugural dissertation, Freie Universität Berlin, Berlin. Journal number 1312, 116–120.
5 *Methods for the Microbiological Examination of Foods.* APHA Inc., Washington D.C.



Appearance of *Shigella spp.* (arrowed) on Hektoen Enteric Agar.

MacConkey Agar No. 3

MacConkey Agar No. 3
Code: CM115

A selective medium giving excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram-positive cocci.

Formula

	grams/litre
Peptone	20.0
Lactose	10.0
Bile salts No. 3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

pH 7.1 ± 0.2

Directions

Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Description

A more selective modification of MacConkey medium which is suitable for the detection and enumeration of coliform organisms and also for the detection and isolation of *Salmonella* and *Shigella species* occurring in pathological and food specimens. Due to the inclusion of a specially prepared fraction of bile salts in addition to crystal violet, the medium gives improved differentiation between coliforms and non-lactose-fermenting organisms whilst Gram-positive cocci are completely inhibited.

This formulation corresponds to that recommended by the American Public Health Association[1] for the direct plating of water samples for coliform bacilli, for the examination of food samples for food poisoning organisms[2] and for the isolation of *Salmonella* and *Shigella species* in cheese.[3]

Rutsch[4] reported that addition of novobiocin to MacConkey Agar improved isolation of *Sh. flexneri* and *Sh. sonnei* from various foods.

The addition of 100 mg of 4-methylumbelliferyl-β-D-glucuronide to one litre of MacConkey Agar detects the enzyme β-glucuronidase.[5] The cleaved 4-methylumbelliferyl moiety is fluorescent at 366 nm. Thus colonies of *Esch. coli* can be detected rapidly in mixed cultures by examining the plate under a uv lamp after overnight incubation at 35°C.[6] However, it should be remembered that other organisms may also be β-glucuronidase-positive.

MacConkey Agar No. 3 was chosen by Rappaport and Henig for the development of Sorbitol MacConkey Agar.[7]

Technique

After inoculation the plates are usually incubated for 18–24 hours at 35°C and for a further 24 hours if non-lactose-fermenting organisms are sought and have not appeared. Lower incubation temperatures may sometimes be used for psychrophilic species. After 18 hours at 35°C, coliforms produce intense violet-red colonies whilst non-lactose fermenters are colourless.

Storage

The dehydrated medium should be stored below 25°C and used before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Escherichia coli ATCC® 25922
Shigella sonnei ATCC® 25931

Negative control:
Enterococcus faecalis ATCC® 29212

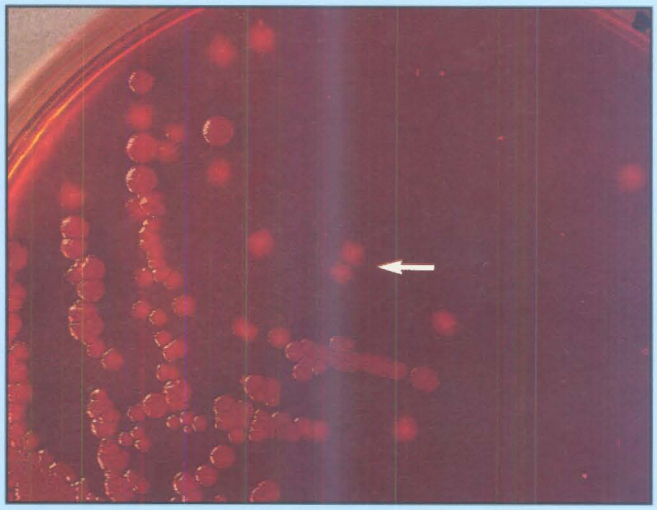
Precautions

Prolonged incubation may lead to confusing results. Do not incubate beyond 48 hours.

Test the medium with a laboratory stock strain of *Shigella species* which is in the R-phase. R-phase shigellae should grow satisfactorily on MacConkey Agar.

References

1 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater.* 15th edn. APHA Inc. Washington DC.
2 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Food.* APHA Inc., Washington DC.
3 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products.* 14th edn. APHA Inc., Washington DC.
4 Rutsch, C. (1987) Inaugural Dissertation, Freie Universität Berlin, Berlin. Journal number 1312, 116–120.
5 Trepeta, A.W. and Edburg, S.C. (1984) *J. Clin. Microbiol.* **19,** 172–174.
6 Maddocks, J.L. and Greenan, M.J. (1975) *J. Clin. Pathol.* **28,** 686–687.
7 Rappaport, F. and Henig, E. (1952) *J. Clin. Path.* **5,** 361.



Appearance of *Shigella spp.* (arrowed) on MacConkey Agar number 3.

Salmonella Shigella Agar (SS Agar)

Salmonella Shigella Agar (SS Agar)
Code: CM99

A differential selective medium for the isolation of Salmonella and some Shigella species from clinical specimens, foods etc.

Formula

	grams/litre
"Lab-Lemco" powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0
pH 7.0 ± 0.2	

Directions

Suspend 63 g in 1 litre of distilled water. Bring to the boil with frequent agitation and allow to simmer gently to dissolve the agar. **Do not autoclave**. Cool to about 50°C, mix and pour into sterile petri dishes.

Description

SS Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella species* from pathological specimens and foods. Gram-positive and coliform organisms are inhibited by the action of the selective inhibitory components, brilliant green, bile salts, thiosulphate and citrate. Addition of Streptomycin to SS Agar has been reported to improve the isolation of Shigella from shrimp and vegetables.[1]

Thiosulphate in combination with iron also acts as an indicator for sulphide production, which is indicated by blackening in the centres of Salmonella colonies.

Technique

Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order to obtain well separated colonies on some part of the plate. Incubate for 18 to 24 hours at 35°C, and subculture on another SS Agar plate.

In parallel with the SS Agar plate, inoculate a tube of Selenite Broth CM395 enrichment medium, incubate for 12 hours at 35°C, and subculture on another SS Agar plate.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive control:
Salmonella enteritidis ATCC® 13076
Shigella sonnei ATCC® 25931
Sh. flexneri ATCC® 12022
Sh. boydii NCTC 11462
Sh. dysenteriae NCTC 9721

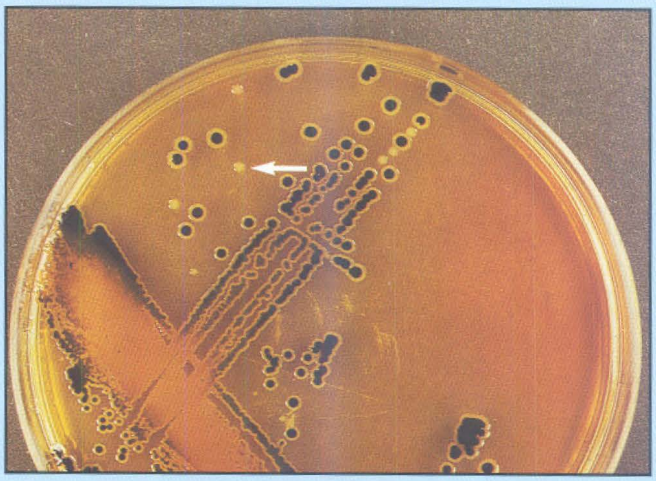
Negative control:
Enterococcus faecalis ATCC® 29212

Precautions

This medium is highly selective and R-strains of shigellae will not grow on it. It is not recommended for the primary isolation of shigellae.[2,3] For this purpose SS Agar (modified) CM533 is preferred.

References

1 Beckers, H.J. and Soentoro, P.S.S. (1989) *Zentralblatt für Bakteriologie Hygiene* **B187,** 261–265.
2 Leifson, E. (1935) *J. Path. Bact.* **40,** 581.
3 Taylor, W.I. and Harris, B. (1965) *Am. J. Clin. Path.* **44,** 476.



Appearance of *Shigella spp.* (arrowed) on Salmonella Shigella Agar (SS agar).

XLD Agar

XLD Agar
Code: CM469

A selective medium for the isolation of salmonellae and shigellae from clinical specimens and foods.

Formula

	grams/litre
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5
pH 7.4 ± 0.2	

Directions

Suspend 53 g in 1 litre of distilled water. Heat with frequent agitation until the medium boils. **Do not overheat**. Transfer immediately to a water bath at 50°C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes which will cause prolonged heating.

Description

Xylose-Lysine-Desoxycholate Agar was originally formulated by Taylor[1] for the isolation and identification of shigellae from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both salmonellae and shigellae.[2] It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of shigellae and salmonellae from non-pathogenic bacteria.

Rapid xylose fermentation is almost universal amongst enteric bacteria, except for members of the *Shigella, Providencia* and *Edwardsiella genera.* Xylose is thus included in the medium so that *Shigella spp.* may be identified by a negative reaction.

Salmonella spp. are differentiated from non-pathogenic xylose fermenters by the incorporation of lysine in the medium. Salmonellae exhaust the xylose and decarboxylate the lysine, thus altering the pH to alkaline and mimicking the *Shigella* reaction. However, the presence of *Salmonellae* and *Edwardsiella spp.* is differentiated from that of shigellae by a hydrogen sulphide indicator.

The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these microorganisms until after the 18 to 24 hour examination for pathogens.

Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliforms without decreasing the ability to support shigellae and salmonellae.

The recovery of *Shigella spp.* has previously been neglected despite the high incidence of shigellosis. This has been due to inadequate isolation media. The sensitivity and selectivity of XLD Agar exceeds that of the traditional plating media, e.g. Eosin Methylene Blue, Salmonella-Shigella, and Bismuth Sulphite Agars, which tend to suppress the growth of shigellae. Many favourable comparisons between XLD Agar and these other media have been recorded in the literature.[4,5,6,7,8,9,10]

The recovery of salmonellae and shigellae is not obscured by profuse growth of other species,[3] therefore XLD Agar is ideal for the screening of samples containing mixed flora and suspected of harbouring enteric pathogens e.g. medical specimens or foods products. Chadwick, Delisle and Beyer[11] recommended the use of this medium as a diagnostic aid in the identification of Enterobacteriaceae.

XLD Agar, in conjunction with MLCB Agar, is specified for use following enrichment culture in Modified Semi-Solid Rappaport Medium (MSRV) when examining faeces for *Salmonella spp.*[12]

Technique

Faeces or rectal swabs may be plated directly[13] or selective enrichment broths may be used prior to streaking out. Selenite broth CM395 or Tetrathionate Broth CM29 may be used for salmonella enrichment.

1 Inoculate the poured, dried plates with a loopful of inoculum either from a suitable enrichment broth, from stool samples or rectal swabs.

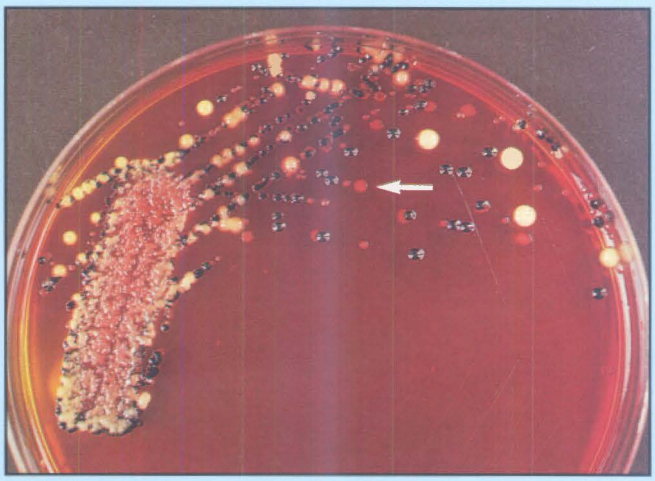
2 Incubate the plates at 35–37°C for 18 to 24 hours.

Colonial Appearance

Organism	Appearance
Salmonella Edwardsiella	Red colonies with black centres
Shigella Providencia H S-negative Salmonella (e.g. *S. paratyphi A*)	Red colonies
Enterobacter *Klebsiella* *Citrobacter* *Proteus* *Serratia*	Yellow, opaque colonies

Note

False positive, red colonies may occur with some *Proteus* and *Pseudomonas species.*



Appearance of *Shigella spp.* (arrowed) on XLD Agar.

Storage Conditions and Shelf Life

Store the dehydrated medium below 25°C and use before the expiry date on the label.

Store the prepared medium at 2–8°C.

Quality Control

Positive controls:
Salmonella typhimurium ATCC® 14028
Shigella sonnei ATCC® 25931
Sh. flexneri ATCC® 12022
Sh. boydii NCTC 11462
Sh. dysenteriae NCTC 9760

Negative control:
Escherichia coli ATCC® 25922

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Modified Desoxycholate-citrate Agar for isolation of shigellae from water

Formula

	grams/litre
Tryptone	20.0
Lactose	10.0
Sodium thiosulphate (pentahydrate)	6.8
Iron III ammonium citrate	800 mgm
Neutral red	30 mgm
Sodium desoxycholate	500 mgm
DL serine	1.0
Agar	14.0
Tetracycline hydrochloride	32 mgm
Distilled water	1000 ml

Reference

The Microbiology of Water 1994 Part 1. Drinking Water. Report on Public Health and Medical Subjects number 71, *Methods for the Examination of Water and Associated Materials.* HMSO, London.

Shigella Toxins

In contrast to enterotoxic *E. coli*, the most outstanding feature of behaviour in infection by any of the four species of Shigella is the invasiveness of the organisms due to similar virulence factors in all species. These virulence factors enable the bacterial cells to adhere to mucosal cells, invade them and then spread laterally to adjacent cells to produce the characteristic pathology. In addition to the virulence factors common to all species, *Shigella dysenteriae* produces shiga toxin. The precise function of this toxin in the overall pathogenic process of Shigella infection is still unclear because mutants that do not produce Shiga toxin are able to invade and kill host cells as readily as those that produce toxin. In addition to being an enterotoxin, Shiga toxin possesses neurotoxic activity and is cytotoxic to animal cells in tissue culture.

Shiga toxin, like the virtually identical verocytotoxin of enterohaemorrhagic strains of *E. coli*, may cause Haemolytic Uraemic Syndrome (HUS) to develop as a complication following dysentery.

Neutralisation tests provide evidence that Shiga toxin is produced by other species of Shigella. A cytotoxin has been detected which can be neutralised by antiserum to Shiga toxin and Shiga toxin-neutralising antibodies have been demonstrated in patients with *Sh. flexneri* or *Sh. sonnei*. If these species do produce Shiga toxin their toxin activity is obviously considerably less than that shown by *Sh. dysenteriae*.

There is evidence that other toxins may be produced by *Shigella spp*. A cytotoxin apparently distinct from Shiga toxin has been detected in disrupted cells of *Sh. sonnei*, *Sh. flexneri*, *Sh. boydii* and *Sh. dysenteriae* grown in iron-depleted medium. For many of the strains tested it was not possible or only partially possible to neutralise the effect with antiserum to Shiga toxin.

Another study has show differences in activity against rabbit ileal segments of Shiga toxin and a cell-free preparation of lysed *Sh. dysenteriae* cells from which Shiga toxin had been removed. Considerable differences in inflammation and tissue necrosis were shown by the two preparations.

A cytolethal distending toxin (CLDT) immunologically similar to CLDT produced by *E. coli* has been detected in culture filtrates of *Sh. dysenteriae* type 2 and *Sh. boydii* type 7.

Production of this cytotoxin was as common as production of Shiga toxin but it occurred in different serogroups.

The relationship of CLDT of cytotoxins (Shiga toxin excluded) detected in other work is unclear.

Strains of *Sh. flexneri* have been shown to produce a heat-stable cell-bound enterotoxin.

These examples have been taken from work conducted some years ago. Currently there is still much to be learnt concerning production of toxins other than Shiga toxin amongst the Shigella species generally. Where cytotoxins unrelated to Shiga toxin have been detected, their role in the pathogenesis of shigellosis has still to be clarified.

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Serology of Shigella

Shigellae are classified into four species groups based on agglutination reactions. These groups correspond to the named species and each group can be subdivided into a number of subgroups based on O antigens as shown in Table 4.

Table 6 – Shigella subgroups

Group	Species	Number
A	*Sh. dysenteriae*	13
B	*Sh. flexneri*	6
C	*Sh. boydii*	18
D	*Sh. sonnei*	1

Agglutination sera are generally produced in rabbits. Division into the subgroups is epidemiologically useful and is usually limited to reference laboratories.

Latex agglutination tests for shigellae have been found unsatisfactory.

Table 7 – Biochemical differentiation of Shigella species

Although Shigella species are differentiated principally by their serological reactions the following differences in biochemical reactions may sometimes be helpful.

	S. dysenteriae	*S. flexneri 01 to 05*	*S. flexneri 06*	*S. boydii*	*S. sonnei*
Gas production from glucose	–	18	–	–	–
Lactose frementation	–	–	–	1	2
Indole production	44	62	0	29	0
Mucate fermentation	–	–	–	–	16
Arginine dihydrolase	–	–	49	18	–
Ornithine decarboxylase	–	–	–	3	99
Sucrose fermentation	–	2	–	–	–*

*Some strains may ferment sucrose late.

The figures indicate the percentage of strains tested that show the reactions.

Table modified from Farmer, J.J. III and Kelly, M.T. *Manual of Clinical Microbiology* 5th Edition, ASM Press, Washington D.C.

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Acknowledgements

My thanks to Mr Eric Griffin, formerly of the Microbiology Department, Royal Hampshire County Hospital, Winchester, UK for production of photographs, except where otherwise acknowledged.

Thanks to Mrs Elaine Nichols and Mrs Jenny Upton of the Quality Assurance Laboratory, Oxoid Limited, Basingstoke for their assistance in providing culture media and test reagents.

Thanks also to Mr David Radcliffe of Oxoid Ltd for setting up the *E. coli* ST toxin enzyme immunoassay tests illustrated on page 52.