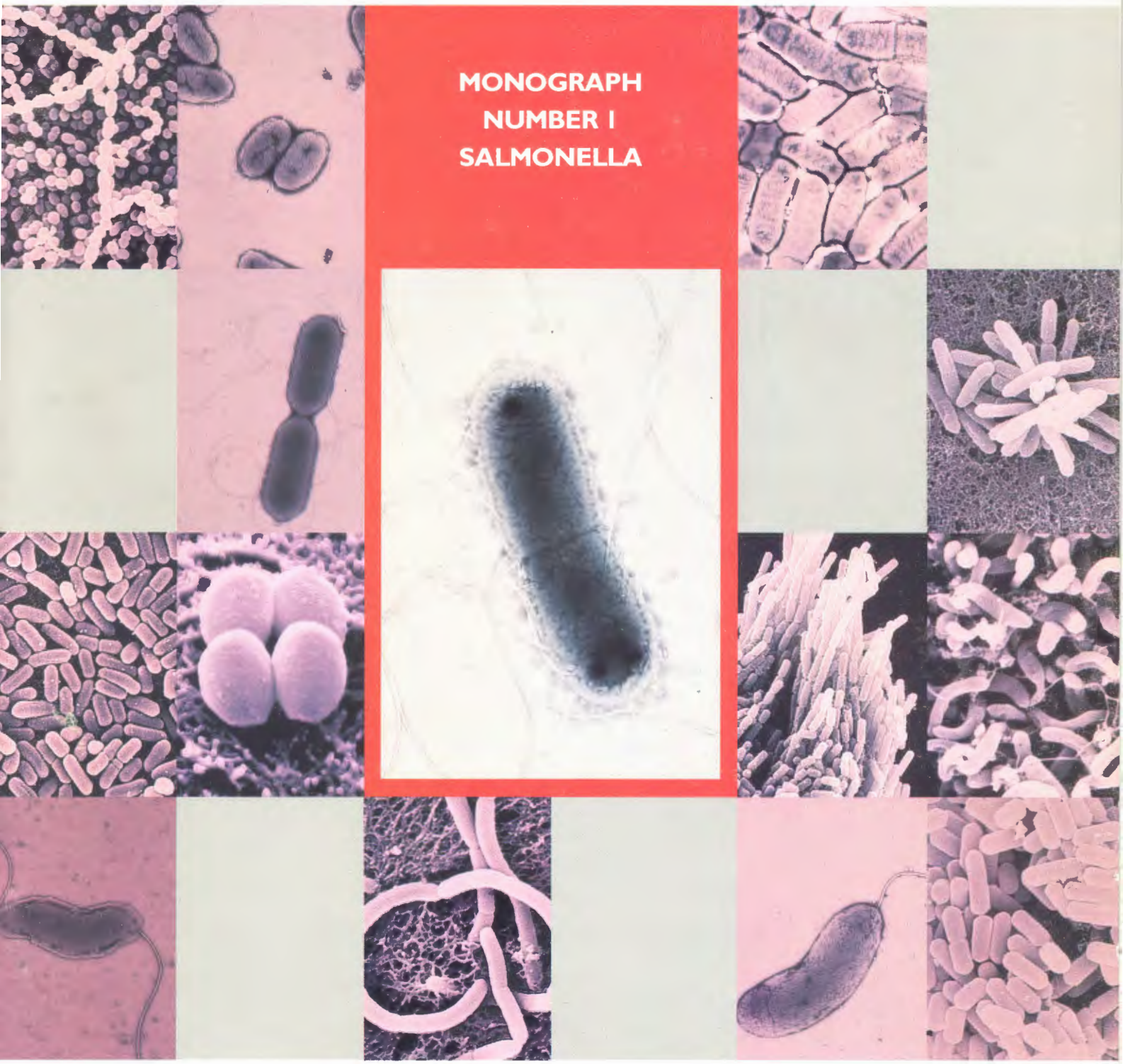
Food-borne Pathogens

MONOGRAPH
NUMBER 1
SALMONELLA



OXOID
SETTING STANDARDS

FOOD-BORNE PATHOGENS

MONOGRAPH NUMBER 1

SALMONELLA

D. E. POST

Technical Support Manager

Foreword

Oxoid microbial culture medium products are inextricably linked with the historical development of bacteriology.

The association can be said to have begun nearly a century and a half ago when Justus von Liebig published his treatise on the extract of meat he was making at the Royal Pharmacy in Munich. The extract, to which he gave the name "extractum carnis" was intended as a nutritional supplement. The medical profession in Germany received it with great enthusiasm and, despite high cost, supply could not meet demand. The extract could not be afforded by the poor because of the expense and short supply. Hope for changing this situation lay with the prospect of being able to use meat taken from the vast herds of cattle roaming the grass lands of South America. Most of the animals were slaughtered only for their hides and fat, the flesh being wasted. Liebig made it known that he would fully assist anyone he judged capable of making his meat extract from this source, to the standard he demanded, at a price that could be afforded by the poor.

In 1861, George Giebert had discussions with Liebig which led to the establishment of a production plant at Fray Bentos in Uruguay. It was a success and, in 1863, the "Fray Bentos Giebert and Company" was established in Antwerp. Control of the production of Liebig's extract was transferred to London in 1865 when Liebig's Extract of Meat Company Limited was formed to take over Giebert's company.

Legal disagreement about the right to use the name Liebig led to the adoption of the trade name LEMCO — taken from the initials of Liebig's Extract of Meat Company. Competitive pressures led to development of an improved product that was given the trade name of OXO in 1899. Production of LEMCO, which was still continuing at the Antwerp factory, was transferred entirely to London at the outbreak of war in 1914, to be continued by the newly formed company of Oxo Limited which, in turn, created Oxoid, a medical division devoted to the sale to physicians of LEMCO meat extract for bacteriological purposes and gland extracts for therapeutic purposes.

Some time later LEMCO was tailored more precisely to the needs of bacteriology and given the name LAB-LEMCO.

It is not surprising that the large staff the company had of chemists and bacteriologists should come together with the consequent eventual development of culture media.

In the early 1920s attempts were made to solubilise dried beef to make an easily digestible product intended for convalescents. The outcome was a peptone coded L37 which with some modification is still marketed as bacteriological peptone.

Further developments, as is so often the case, arose out of necessities imposed by warfare. As part of the war effort the company was producing field rations for the forces. This meant that many bacteriological tests were performed on these products before their release for consumption.

As a result large volumes of culture media were required and the company formulated and manufactured them wherever possible using ingredients manufactured in house.

It quickly became apparent that much time and labour could be saved if the media could be available ready-mixed in a dry form. Thus the first complete and dehydrated media were produced by Oxoid for domestic expediency to be used in controlling the bacteriological quality of food.

Sales of the gland extracts declined and when the Oxoid pharmaceutical department closed in 1957 business was switched to the sale of culture media, the first of which had been available since 1948.

During the entire period of the Company's development outlined above the science of bacteriology was itself evolving with considerable speed. Early observers of microscopic life forms including Needham (1745) had recognised the need for the preparation of suitable nutrient fluids for their growth but there was a lack of uniformity in the methods followed.

The study of nutrient media possessing more exact composition was initiated by Pasteur in 1860. Cohn developed this work and published the formula for his "normal bacterial liquid" in 1870. Klebs noted Needham's early observations that saprophytic and putrefactive bacteria grew particularly well in a watery extract of meat and used a culture fluid of this nature when he commenced study of pathogenic bacteria in 1871.

Nageli first described "peptone" in 1880. He has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein. Robert Koch quickly took up this development and in 1881 described his culture medium which was made from an aqueous meat extract to which was added peptone and sodium chloride. To this day this simple formula is the basic one for culture media.

In the following year (1882) Heuppe described the labour saving convenience of substituting commercial meat extract for Koch's watery extract of fresh meat. By 1902 an American text book of bacteriology was recommending the use of LEMCO for this purpose. This is quite possibly the first record of exporting culture media ingredients by the company.

It will be seen that the business of manufacturing dehydrated culture media was a natural consequence of the converging commercial activities of Oxoid and the development of the science of microbiology. The early history explains why Unipath is one of the very few producers of culture media that actually manufactures its own extracts and hydrolysates.

In 1968 Liebig Extract of Meat Company merged with Brooke Bond Limited. The merged company was given the name Brooke-Bond Oxo and trade in culture media continued under Oxoid Limited.

In 1984 Brooke-Bond Oxo was purchased by Unilever Plc and for the first time in its history Oxoid was separated from Oxo. This prepared the way for all Unilever's medical products companies to be relaunched under a single international corporate identity, UNIPATH.

Culture media products continue to be marketed under the Oxoid trade mark. It is particularly fitting in this publication addressed to food microbiologists to note that the first intensive work on formulating complete Oxoid culture media more than half a century ago was for use in the microbiological examination of food.

The expertise gained over more than a century of manufacturing extracts and hydrolysates is present in the wide range of quality products available today.

Contents

Acknowledgements

I wish to thank Maryalice Fancisco formerly of Marketing Department, Unipath Basingstoke for providing information used in the compilation of tables in this publication.

The phototgraphs of Salmonella growth on the various culture media were produced by Mr Eric Griffin of the Microbiology Department, Royal Hampshire County Hospital, Winchester UK. My thanks to him and the staff of the Microbiology Department for their assistance.

Introduction

Despite the controls that have already been put into place, Salmonella infection arising from contaminated food continues to be an immense problem with millions of cases occurring annually throughout the world. In addition to the misery caused, financial loss is enormous. Todd estimated in 1989 that the cost of food-borne salmonellosis in the United States alone amounted to $3,990,000,000. Detection of Salmonella before contaminated foods can be consumed is therefore an essential feature of safeguarding public health and incidentally preserving the reputations and fortunes of food manufacturers and processors.

This publication is intended to serve as a guide to the Oxoid culture media that are available for this extremely important aspect of food microbiology.

Salmonella Isolation Methods

Considerable effort has been devoted to defining the conditions required for isolation of Salmonella and standardised procedures are specified by a number of bodies. These generally relate to all aspects of methodology including the culture media to be used. Standards Bodies and media they specify for Salmonella detection are named in Table 1. However, there is no single "correct" methodology that can be applied to all foods and circumstances. Successful isolation may require the employment of culture media additional to the minimum specified and standard methodological procedures may need to be augmented.

TABLE 1 — Some Regulatory Bodies that specify detection procedures for Salmonella and the culture media to be used. The codes for the Oxoid dehydrated culture media that are available from Unipath are in parentheses.

Body	Culture Media		
	PRE-ENRICHMENT	ENRICHMENT	PLATING
ISO	Buffered Peptone Water (CM509)	Rappaport-Vassiliadis (RV) Broth (CM669) Selenite Cystine Broth (CM699)	Brilliant Green Agar (Edel & Kampelmacher) (CM329) Any other solid selective medium*
APHA	Lactose Broth (CM137)	Selenite Cystine Broth (CM699) Tetrathionate Broth (USP) (CM671)	SS Agar (CM99) Bismuth Sulphite Agar (CM201) Hektoen Agar (CM419)
AOAC /FDA	Lactose Broth (CM137) Tryptone Soya Broth (CM129) Nutrient Broth (Lab-Lemco Broth CM15)**	Selenite Cystine Broth (CM699) Tetrathionate Broth (USP) (CM671)	Brilliant Green Agar (CM263) Hektoen Agar (CM419) XLD Agar (CM469) Bismuth Sulphite Agar (CM201)
IDF	Buffered Peptone Water (CM509) Distilled Water Plus Brilliant Green 0.002%**	Muller-Kauffman Tetrathionate Broth (CM343) Selenite Cystine Broth (CM699)	Brilliant Green Agar (Edel & Kampelmacher) (CM329) Bismuth Sulphite Agar (CM201)
BSI	Buffered Peptone Water (CM509)	Rappaport-Vassiliadis (RV) Broth (CM669) Selenite Cystine Broth (CM699)	Brilliant Green Agar (Edel & Kampelmacher) (CM329) Any other solid selective medium*

* The choice of the second medium is discretionary unless a specific medium is named in an International Standard relating to the product to be examined.
** The choice of pre-enrichment medium is dependent on the product under examination. The standard methods of the appropriate body should be consulted for details.

Detection

Salmonella detection is still highly dependent on employing appropriate culture media. Isolation is a complex procedure which involves many interacting factors of which quality of the culture medium is only one. Methods and media must be capable of enabling growth to occur from extremely low initial cell numbers. In addition the cells may be stressed or have sustained actual damage during processing of the food. Finally, the injured cells are frequently accompanied by competing organisms which are present in numbers thousands of times greater. The essential features of any cultural procedure therefore are that the media in use should initially be capable of resuscitating Salmonella and then conferring on them a selective advantage. This will enable them to compete successfully so that they may reproduce sufficiently to be detected amongst the accompanying flora. Detailed choice of the most appropriate culture media may be considerably influenced by the food under investigation and the characteristics of the indigenous microorganisms. These two important considerations are to a great extent responsible for the number of media formulae that have been devised in the quest for optimum performance.

Standardised isolation procedures drawn up by expert bodies state the culture media to be used. These procedures have generally been established after collaborative testing and many have international recognition. However, for reasons already stated there is no single "correct" methodology that can be applied to all foods. The media described in the following pages have all been designed for isolation of Salmonella. Where standardised procedures are being followed, alternative media may be used in addition to, but not in place of, the media specified by the Standard Body.

Isolation procedures as typified by BSI/ISO and FDA/AOAC BAM are shown in figures 1 and 2.

FIGURE 1
BSI/ISO Salmonella Isolation Procedure

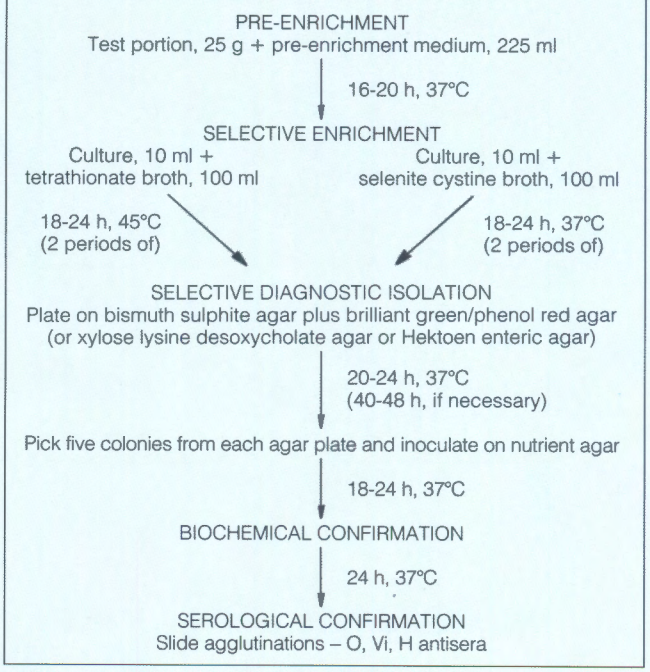
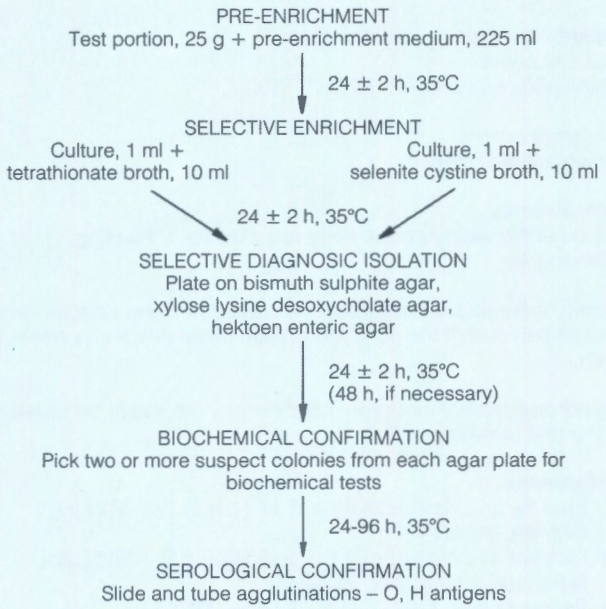


FIGURE 2

FDA/AOAC BAM Salmonella Isolation Procedure

Conventional Cultural Enrichment (FDA/BAM/AOAC)



Resuscitation and pre-enrichment

Satisfactory resuscitation and pre-enrichment generally requires a nutritious non-selective medium. Buffered peptone water and lactose broth are commonly used but other nutrient media such as tryptone soya and nutrient broths may also be employed. Oxoid Lab-Lemco broth conforms to the formula specified for nutrient broth by the Association of Official Analytical Chemists but note that the pH is higher than 6.8 ± 0.2 specified by AOAC. It may be necessary to neutralise any toxicity contributed by the sample under test. In practice, the dilution afforded by sample preparation is generally sufficient for most foods but there are exceptions which require special treatment, for example chocolate is naturally toxic to injured Salmonella and it is recommended that casein should be present in pre-enrichment medium when testing chocolate products.

Lactose Broth may be unsuitable as a pre-enrichment medium when applied to samples that contain a high population of lactose fermenting organisms because the consequent acid production may reduce the pH of the medium to levels that prevent growth of damaged Salmonella.

Non-selective pre-enrichment broths are suitable for most foods but problems of overgrowth may occur where large numbers of Gram-positive bacteria are present. In these circumstances addition of 0.002% of brilliant green or 0.01% of malachite green to buffered peptone water may be beneficial.

Distilled water containing 0.002% of brilliant green may be used for dried milk and milk products, the samples themselves contributing the nutrients required.

Pre-enrichment media and the commodities it is recommended they be used with are listed in table 2.

TABLE 2

Selection of Pre-enrichment Media	
Medium	*Commodity*
Buffered Peptone Water (BPW)	General Purpose
BPW + Casein	Chocolate etc.
Lactose Broth	Egg and Egg Products; Frog Legs; Food Dyes pH>6
Lactose Broth + Tergitol 7 or Triton X-100	Coconut; Meat; Animal Substances – Dried or Processed
Lactose Broth + 0.5% Gelatinase	Gelatin
Non-Fat Dry Milk + Brilliant Green	Chocolate; Candy and Candy Coatings
Tyrptone Soya Broth	Spices; Herbs; Dried Yeast
Tryptone Soya Broth + 0.5% Potassium Sulphate	Onion and Garlic Powder etc.
Water + Brilliant Green	Dried Milk

Buffered Peptone Water

Code: CM509

A pre-enrichment medium to be used prior to selective enrichment for the isolation of Salmonella species from foods.

Formula (grams per litre)

Peptone	*10.0*
Sodium chloride	*5.0*
Disodium phosphate	*3.5*
Potassium dihydrogen phosphate	*1.5*

pH 7.2 ± 0.2

Directions

Add 20g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

It is very important that the water used is of high quality. Distilled water should have a low mineral content/conductivity.

Description

Oxoid Buffered Peptone Water CM509 may be used as a pre-enrichment medium, prior to selective enrichment in the isolation of salmonellae from foods. It also provides conditions for resuscitation of cells that have been injured by processes of food preservation.

It was noted by Edel and Kampelmacher[1] that sublethal injury to salmonellae may occur in many food processes. In a survey involving isolation of salmonellae from meat that had been artificially contaminated with sublethally injured organisms, pre-enrichment in buffered peptone water at 37°C for 18 hours before selection in brilliant green-tetrathionate bile broth showed superior results compared with a direct selection method.

Pietzsch[2] found that isolation of salmonellae was much improved by pre-enrichment of egg samples in buffered peptone water at 37°C for 18 hours followed by incubation of 10ml of this sample in 100ml Selenite Cystine Broth CM699 or Muller-Kauffmann Tetrathionate Broth CM343 at 43°C for 48 hours.

Sadovski[3] reported that, in experiments involving isolation of salmonellae from frozen vegetables, the rapid drop in pH when using lactose broth[4] as a pre-enrichment medium was detrimental to the recovery of salmonellae. This was due to the enhanced sensitivity to low pH of freeze-injured salmonellae which may contaminate frozen vegetables. Pre-enrichment with buffered peptone water maintained a high pH over a period of 24 hours incubation. Vegetable tissue has a low buffering capacity and the medium overcame this problem.

A shortened enrichment time of 6 hours was investigated[6] but in circumstances where heavily contaminated materials were examined, the addition of 0.1g of malachite green per litre of buffered peptone water was advised.

The addition is important where small numbers of Salmonella may have their generation time increased because of competitive growth and may not reach the minimum number for successful isolation.

Technique for the isolation of salmonellae[5]

1. Add 10g of sample to 50ml of Buffered Peptone Water CM509 and mix thoroughly.
2. Incubate at 35°C for 18 hours.
3. Add 10ml of incubated BPW to 100ml of Muller-Kauffmann Tetrathionate Broth CM343.
4. Incubate at 43°C.
5. Subculture to Brilliant Green Agar CM263 or Brilliant Green Agar (Modified) CM329, after 24 and 48 hours incubation.
6. Incubate the Brilliant Green Agar plates at 35°C for 18 hours.
7. Examine the plates for colonies of *Salmonella* species.

Storage conditions and Shelf life

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

Store prepared medium at 2-8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Uninoculated medium.

Precautions

Observe the safety precautions required for cultivating salmonellae.

Liquid cultures are more infective than plates and special care should be taken if the 43°C incubation takes place in a water bath.

Do not use malachite green if *Salmonella typhi* may be present in the test material.

References

1 Edel W. and Kampelmacher E.H. (1973) *Bull. Wld. Hlth. Org.* **48,** 167-174.
2 Pietzsch O., Kretschmer F.J. and Bulling E. (1975) *Zbl. Bakt. Abt. I. Orig.* **232,** 232-246.
3 Sadovski A. Y. (1977) *J.Fd. Technol.* **12,** 85-91.
4 Angelotti R. (1963) *'Microbiological Quality of Foods' Academic Press, New York.* p.149.
5 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. A.P.H.A. Inc. Washington D.C.*
6 van Schothorst M. and Renaud A.M. (1985) *J. Appl. Bact.* **59,** 223-230.

'Lab-Lemco' Broth

Code: CM15

A Nutrient Broth for general bacteriological use, and for the examination of water, sewage, dairy products and food by American standard methods.

Formula (grams per litre)

'Lab-Lemco' powder	*3.0*
Peptone	*5.0*

Final pH 7.4 ± 0.2

Directions

Dissolve 8g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description

'Lab-Lemco' Broth is a general purpose liquid medium used for the examination of water and dairy products.[1,2]

It is used as a nutrient meat extract broth for general bacteriology.

The formula conforms to that for Nutrient Broth specified by the Association of Official Analytical Chemists for pre-enrichment of food samples undergoing examination for detection of Salmonella (see table 1).

For pre-enrichment 25g samples are added to 225ml of broth. The specific methods given in the AOAC Bacteriological Analytical Manual should be referred to for samples that cannot be analysed on an exact weight basis.

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:
Staphylococcus aureus ATCC 25923
Escherichia coli ATCC 25922

Negative control:
Uninoculated medium.

Precautions

'Lab-Lemco' broth is slightly more alkaline than the medium used in the APHA publications (pH 6.8).

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* (1978) Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.

Lactose Broth

Code: CM137

A liquid medium for use in the performance or confirmation of the Presumptive Test for Coliforms in water, milk, etc and for use as a pre-enrichment broth for Salmonella.

Formula (grams per litre)

'Lab-Lemco'powder	*3.0*
Peptone	*5.0*
Lactose	*5.0*

Final pH 6.9 ± 0.2

Directions

Dissolve 13g in 1 litre of distilled water and distribute into containers with fermentation tubes (Durham). Sterilise by autoclaving at 121°C for 15 minutes.

Description

Lactose broth is recommended for use in the presumptive identification of coliform organisms in milk, water and foods as specified by the American Public Health Association.[1,2,3]

Tubes of Lactose Broth are inoculated with dilutions of the samples and incubated at 35°C. Examination for gas formation is carried out after 24 and 48 hours incubation. This presumptive evidence of coliform organisms must be confirmed by further tests.

Lactose Broth is also specified by APHA and by the Association of Official Analytical Chemists (AOAC) for pre-enrichment culture of Salmonella[3,4] (see Table 1).

For pre-enrichment of food samples 25g units of sample are added to 225ml of broth.

The specific AOAC method should be referred to for samples that cannot be analysed on an exact weight basis.[4]

Storage conditions

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
Enterobacter aerogenes ATCC 13048

Negative control:
Uninoculated medium.

References

1 *American Public Health Association* (1979) Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Washington DC.
2 *American Public Health Association* (1980) Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington DC.
3 *American Public Health Association* (1976) Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington DC.
4 "Association of Official Analytical Chemists" *F.D.A. Bacteriological Analytical Manual* 6th Edition (1989) AOAC Arlington Virginia USA.

Tryptone Soya Broth
Soybean Casein Digest Medium USP.

Code: CM129

A highly nutritious general purpose medium for the growth of bacteria and fungi.

Formula (grams per 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*

Final pH 7.3 ± 0.2

Directions

Dissolve 30g in 1 litre of distilled water and distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

Description

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 one of a number of media recommended by AOAC for pre-enrichment culture of Salmonella (see Table 1).

For pre-enrichment of food samples 25g units of sample are added to 225ml of broth. The specific methods given in the AOAC Bacteriological Analytical Manual should be referred to for samples that cannot be analysed on an exact weight basis.[1]

References

1 "Association of Official Analytical Chemists" *F.D.A. Bacteriological Analytical Manual* 6th Edition (1989) AOAC Arlington Virginia USA.

Selective Enrichment

Selective enrichment broths are employed for the purpose of increasing the Salmonella population while at the same time inhibiting multiplication of other organisms in the food sample.

A variety of inhibitors is in use, the most widely used of which are bile, tetrathionate, sodium selenite and either brilliant green or malachite green dyes. Two or more of these inhibitors may be used in combination and additionally media may be supplemented with antibiotics, commonly novobiocin. Activity of inhibitory agents may be further enhanced by incubation of the enrichment culture at higher temperatures, usually between 41°C and 43°C. A more selective medium is generally preferable if the amount of accompanying flora is high. Investigations should ideally employ more than 1 enrichment broth to ensure that the media in use are capable of supporting growth of the widest possible range of serovars. All the variations of selenite broth are suitable for most serovars including *S. typhi, S. dublin* and *S. choleraesuis*. Mannitol selenite broth is reported to be superior to selenite F broth for the growth, of *S. typhi*. The addition of cystine to selenite broth enhances Salmonella growth, probably by reducing the selective activity of sodium selenite. The formulation of selenite cystine broth corresponds to that recommended by the AOAC and is commonly used in food microbiology.

Selenite broths are not highly selective and are probably best used in conjunction with tetrathionate broth where it is necessary to investigate for the widest possible range of serovars in samples with a high population of accompanying flora.

Tetrathionate broths depend for their selectivity on the ability of thiosulphate and tetrathionate in combination to suppress the growth of coliform organisms. Salmonella species possess the enzyme tetrathionate reductase and consequently are able to grow in the medium; unfortunately this enzyme is also possessed by Proteus species. In situations where overgrowth of Proteus may interfere with the detection of Salmonella, increased incubation temperature serves to limit the growth of Proteus. Alternatively the addition of novobiocin to tetrathionate broth may be beneficial. Muller-Kauffmann tetrathionate broth is an improved medium which contains carefully selected brilliant green and bile to improve selectivity and suppress the growth of Proteus. However, it should not be used if *S. typhi* is suspected.

The original formula of Rappaport broth was developed to exploit important characteristics of Salmonella species which are not shared to the same extent by other Enterobacteriaceae. It contains a high concentration of Magnesium chloride to reduce water activity (aw) and has a pH of 5.2. Malachite green is included to further enhance selectivity. Since the medium was first described, the formula has undergone a number of modifications which, in conjunction with changes in incubation temperature, has increased its sensitivity.

Semi-solid Rappaport medium is a further recent development. Motile salmonellae migrate through the semi-solid gel ahead of competing organisms. The medium is not suitable for the detection of non-motile strains of Salmonella.

Selenite Broth Base (Lactose)

Code: CM395

Used with Sodium biselenite for the preparation of Selenite F broth for selective enrichment of Salmonella from faeces and food products.

Formula (grams per 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 5cm. 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.

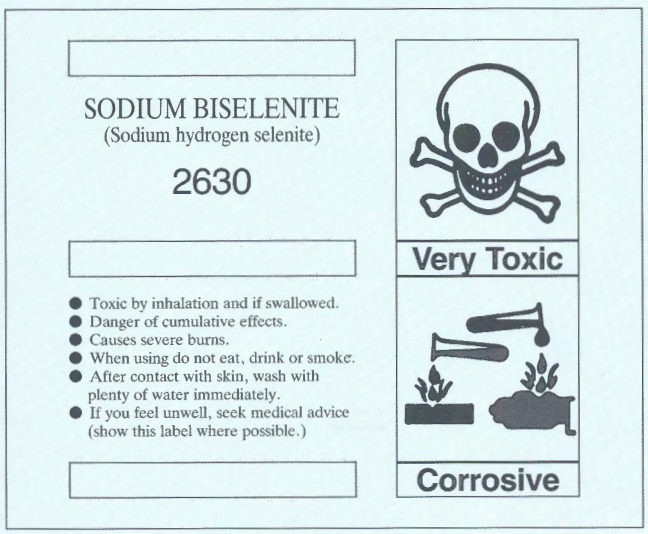
Thompson[7] reported miscarriages and possible teratogenic effects on pregnant laboratory assistants which may have been caused by ingested sodium biselenite. Oxoid therefore removed this substance from the powdered medium to remove the risk of inhalation.

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

Sodium Biselenite (Sodium Hydrogen Selenite)
Code: L121

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

Keep tightly closed, in a cool dry place away from bright light.



Description
Klett[1] first demonstrated the selective inhibitory effects of selenite and Guth[2] used it to isolate *Salmonella typhi*. It was twenty years later before Leifson[3] fully investigated selenite and promoted wide use of the medium.

Selenium toxicity to certain micro-organisms is not fully understood but it is suggested that it reacts with sulphur and sulphadryl groups in critical cell components[4,5].

Proteus and *Pseudomonas* species appear to be resistant to its effects[4]. Lactose is added as a fermentable carbohydrate to prevent a rise in pH 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 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[6].

Technique
For routine purposes Selenite F 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 agars 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 on to 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 15ml of saline in a wide-necked 1oz. bottle, emulsify, separate the debris by slowly pressing a plug of cotton-wool down through the suspension. Withdraw approximately 1ml of the supernatant and inoculate 10ml of Selenite Broth.

Harvey & Scott Thomson[2] showed that incubation of the selenite broth at 43°C facilitated the isolation of *Salmonella paratyphi B* from faeces. They recommend the use of this principle for the examination of sewage and river water containing large numbers of other bacteria that preferred a lower temperature for growth. The authors also suggested that the procedure was of value for all salmonellae except *Salmonella typhi*. For urines, the broth should be made double strength and inoculated with its own volume of the specimen.

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[10].

Take subcultures of broth from the upper third of the broth column, which should be at least 5cm in depth.

Observe the precautionary comments made about Sodium biselenite in the Directions.

References

1 Klett A. (1900) *Zeitsch. fur Hyg. und Infekt.*, **33,** 137-160.
2 Guth F. (1916) *Zbl. Bakt. I. Orig.*, **77,** 487-496.
3 Leifson E. (1936) *Amer. J. Hyg.*, **24,** 423-432.
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6 Fagerberg D.J. and Avens J.S. (1976) *J Milk, Food Technol.*, **39,** 628-646.
7 Thompson (1970) *Lancet i.*, 518-519.
8 Harvey R.W.S. and Scott T. (1953) *Mon. Bull. Min. Hlth. & PHLS.*, **12,** 149-150.
9 Harvey R.W.S. and Price T.H. (1979) *J. Appl. Bact.*, **46,** 27-56.
10 Chattopadhyay W. and Pilford J.N. (1976) *Med. Lab. Sci.*, **33,** 191-194.

Selenite Broth Base (Mannitol)

Code: CM399 – Sodium Biselenite Code: L121

(See Selenite Broth Base)

A modification of Selenite F Broth especially recommended for the enrichment of salmonellae.

Formula (grams per litre)

Bacteriological peptone	*5.0*
Mannitol	*4.0*
Sodium phosphate	*10.0*
pH 7.1 ± 0.2	

Directions

Add 19 grams to 1 litre of distilled water to which 4 grams of sodium biselenite L121 has been added.

Warm to dissolve, mix well and fill out into containers to a depth of 5cm. Sterilize 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 to this medium separately[3].

Description

This medium is similar to the modification of Leifson[1] enrichment medium described by Hobbs & Allison[2] for the isolation of *Salmonella typhi* and *Salmonella paratyphi B.*

Hobbs & Allison[2] compared two sets of selenite media, one containing lactose and the other mannitol. Of 38 positive stools *S. typhi.* was subcultured from both media in 32 instances, from the mannitol selenite alone in 5 instances and from the lactose selenite alone, once. Comparisons showed that the mannitol selenite broth was superior to three other liquid media in its selective value for *S. typhi* and that it was as good as tetrathionate for the isolation of *S. paratyphi B.*

Technique

Subcultures from this selective, enrichment broth can be made to any combination of greater and lesser inhibitory selective agars for Enterobacteriaceae.

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

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 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.
Mannitol fermentation by Salmonella helps correct the alkaline pH swing, which can occur during incubation.
Take subcultures from the upper third of the broth column, which should be at least 5cm in depth.

References

1 Leifson E. (1936) *Am. J. Hyg.*, **24**(2), 423-432.
2 Hobbs Betty C. and Allison V.D. (1945) *Mon. Bull. Min. Hlth. Pub. Hlth. Lab. Serv.*, **4,** 12-19.
3 Thompson (1970) *Lancet i*, 518-519.

Selenite Cystine Broth Base

Code: CM699

An enrichment medium for the isolation of salmonellae from faeces and food products.

Formula (grams per litre)

Tryptone	*5.0*
Lactose	*4.0*
Disodium phosphate	*10.0*
L-Cystine	*0.01*

pH 7.0 ± 0.2

Directions

Dissolve 4g of Sodium biselenite L121 in 1 litre of distilled water and then add 19g of Selenite Cystine Broth Base CM699. Warm to dissolve and dispense into containers to a depth of at least 60mm. Sterilize 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 micro-organisms, 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 of the American Public Health Association[5,6]. It also complies with the requirements of the United States Pharmacopoeia[7].

Technique

The proportion of sample in the enrichment broth should not exceed 10-20% (1 or 2 grams in 10-15ml). 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. Some workers have recommended that 43°C be used[8,9].

Subculture to any combination of greater and lesser inhibitory, selective agars for Enterobacteriaceae.

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

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 a 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[10].
Take subcultures of broth from the upper third of the broth column, which should be at least 5cm in depth.

References

1 Leifson E. (1936) *Am. J. Hyg.*, **24**(2), 423-432.
2 North W.R. and Bartram M.T. (1953) *Appl. Microbiol. I.*, 130-134.
3 Fricker C.R. (1987) *J. Appl. Bact.*, **63,** 99-116.
4 Association of Official Analytical Chemists (1978) *Bacteriological Analytic Manual. 5th Edn. AOAC. Washington DC.*
5 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA. Inc. Washington DC.*
6 American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*
7 United States Pharmacopoeia XXI (1980) *Microbial Test Limits.*
8 Harvey R.W.S. and Scott T. (1953) *Mon. Bull. Min. Hlth. & PHLS.*, **12,** 149-150.
9 Harvey R.W.S. and Price T.H. (1979) *J. Appl. Bact.*, **46,** 27-56.
10 Chattopadhyay W. and Pilford J.N. (1976) *Med. Lab. Sci.*, **33,** 191-194.

Tetrathionate Broth Base

Code: CM29

Formula (grams per litre)

'Lab-Lemco' powder	*0.9*
Peptone	*4.5*
Yeast extract	*1.8*
Sodium chloride	*4.5*
Calcium carbonate	*25.0*
Sodium thiosulphate	*40.7*

pH 8.0 ± 0.2

Directions
Add 77g to 1 litre of distilled water and bring to the boil. Cool below 45°C and add 20ml of iodine solution. Mix well and tube in 10ml quantities. The prepared base will keep for several weeks at 4°C but should be used soon after the addition of the iodine solution.

Iodine Solution

Iodine	*6 grams*
Potassium iodide	*5 grams*
Distilled water	*20ml*

Description
Tetrathionate Broth is recommended for the selective enrichment method of isolating *Salmonella typhi* and other salmonellae from faeces, sewage, etc.

Organisms which reduce tetrathionate, such as salmonellae, flourish in the medium whilst many faecal organisms are inhibited[1]. Members of the *Proteus* group reduce tetrathionate and may consequently impair the value of this medium for the isolation of salmonellae; this disadvantage of the medium is largely overcome by the addition of 40μg of novobiocin to each millilitre of the incomplete medium before the addition of iodine[2,3].

Technique
Inoculate the broth with about 2 grams of the specimen and mix thoroughly to disperse particulate matter. A loose cotton-wool plug may be passed down through the inoculated medium in order to carry gross particles to the bottom of the tube. Incubate for 12 to 24 hours at 35°C and then subculture on Bismuth Sulphite Agar CM201, SS Agar CM99 or Desoxycholate Citrate Agar (Hynes) CM227, etc.

The complete medium (with added iodine) should be used the same day as it is prepared, but the sterilized basal medium will keep for many weeks at 4°C. Jeffries[2] showed that novobiocin, at a concentration of 40μg/ml in the medium, remained stable for at least 48 hours at 35°C, and for one month at room temperature.

This medium is frequently used in parallel with Selenite Broth Base CM395.

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 (without iodine solution) at 2-8°C

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

References

1 Knox R., Gell P.G.H. and Pollock M.R. (1942) *J. Path. Bact.*, **54,** 469-483.
2 Jeffries L. (1959) *J. Clin. Path.*, **12,** 568-571.
3 Buttiaux R., Catsaras M. and Verdant M. (1961) *Ann. Inst. Pasteur da Lille*, **12,** 13-18.

Tetrathionate Broth (USA)

Code: CM671

An American formulation which complies with the description given in the US Pharmacopoeia for the enrichment of specimens undergoing examination for salmonellae.

Formula (grams per litre)

Casein peptone	*2.5*
Meat peptone	*2.5*
Bile salts	*1.0*
Calcium carbonate	*10.0*
Sodium thiosulphate	*30.0*

pH 8.0 ± 0.2

Directions

Suspend 46 grams in 1 litre of distilled water and bring to the boil. Cool to below 45°C and add 20ml of iodine-iodide solution immediately before use. Mix continuously whilst dispensing 10ml volumes into sterile tubes. Use the complete medium (with added iodine) on the day of preparation.

Iodine-Iodide Solution

Iodine	*6 grams*
Potassium iodide	*5 grams*
Distilled water	*20ml*

Note

The base may be prepared beforehand and kept for several weeks at 4°C. Iodine-iodide solution can then be added at the time of use to the quantity of medium needed.

Description

Tetrathionate Broth (USA) CM671 complies with the description given in the United States Pharmacopoeia.

Tetrathionate broth is specified by the 15th edition of Standard Methods for the Examination of Water and Waste Water[2] and Compendium of Methods for the Microbiological Examination of Foods[3] for the enrichment of specimens undergoing examination for salmonellae.

The selectivity of the medium depends on the ability of thiosulphate and tetrathionate in combination to suppress commensal coliform organisms[4]. Organisms which possess the enzyme tetrathionate reductase grow in the medium. *Salmonella* and *Proteus* species possess the enzyme; *Escherichia coli* and shigellae do not.

Proteus can be suppressed by adding 40μg per ml of novobiocin[6] to the incomplete medium before the addition of iodine.

Bile salts are present to inhibit those organisms which do not live in the intestine.

Brilliant Green 0.001% w/v can be added to the broth[1] but it should be remembered that *Salm. typhi* and some other salmonellae are inhibited by this compound.

The role of calcium carbonate is to neutralize the acidic tetrathionate decomposition products.

Technique

Inoculate the broth with 1-2 grams of the specimen and mix thoroughly to disperse the sample.

Incubate at 35°C and subculture after 18-24 hours to XLD Agar CM469, SS Agars CM99 or CM533, Bismuth Sulphite Agar CM201, or similar selective/indicator media for salmonella isolation.

Storage conditions and Shelf life

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

Store the base broth at 2-8°C. Use the medium immediately after adding the iodine solution.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922

References

1 United States Pharmacopoeia XXI (1985) *Microbial Limit Tests. Rockville, Md.*
2 American Public Health Association (1980) *Standard Methods for the Examination of Water and Wastewater. 15th Edn. APHA Inc. Washington D.C.*
3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington D.C.*
4 Pollock M.R. and Knox R. (1943) *Biochem. J.*, **37,** 476-481.
5 Papavassiliou J., Samaraki-Lyberopoulou V. and Piperakis G. (1969) *Can. J. Microbiol.*, **15,** 238-240.
6 Jeffries L. (1959) *J. Clin. Path.*, **12,** 568-571.

Muller-Kauffmann Tetrathionate Broth Base

Code: CM343

An improved enrichment medium for the isolation of salmonellae and the suppression of Proteus species.

Formula (grams per litre)

Tryptone	*7.0*
Soya peptone	*2.3*
Sodium chloride	*2.3*
Calcium carbonate	*25.0*
Sodium thiosulphate	*40.7*
Ox bile	*4.75*

pH 8.0 ± 0.2

Directions

Suspend 82 grams in 1 litre of distilled water and bring to the boil. Cool below 45°C and add, just prior to use, 19ml of iodine solution and 9.5ml of a 0.1% brilliant green solution. Mix well and fill out into sterile tubes or flasks.

Iodine Solution

Iodine	*20 grams*
Potassium iodide	*25 grams*
Distilled water to	*100ml*

Dissolve the potassium iodide in approximately 5ml of distilled water, add the iodine and gently warm the solution to completely dissolve it. Make up the volume to 100ml with distilled water.

Brilliant Green Solution

Brilliant Green (BDH or Chroma)	*0.1 grams*
Distilled water	*100ml*

Add the brilliant green to the distilled water and shake to dissolve the dye. Heat the solution to 100°C for 30 minutes and shake from time to time whilst cooling, to ensure that the dye has completely dissolved. Store in a brown glass bottle or away from light.

Description

Muller[1] developed this medium in 1923. It was later modified by Kauffmann[2,3] with the addition of brilliant green and ox bile to suppress commensal organisms and thus improve the isolation of salmonellae.

The brilliant green dye used in the medium has been shown to be critical and Chroma or BDH brands should be used. It is essential that the dye is added as directed because heating the brilliant green or attempting to incorporate it in the basal medium seriously impairs its selective action.

The addition of novobiocin at 4mg per litre of broth was described by Jeffries[4] to suppress the growth of *Proteus* species.

Muller-Kauffmann Tetrathionate Broth should not be used if *Salmonella typhi* is suspected.

Muller-Kauffmann Tetrathionate Broth was used in a large scale investigation between nine laboratories in eight different countries[5].

Incubation of Muller-Kauffmann broth at 43°C was shown to be essential in this trial and the technique used for enrichment of the salmonellae is as follows:

Add approximately 10 grams of sample to 100ml of Muller-Kauffmann broth. Shake vigorously and immediately place the flasks of medium in a 45°C water-bath for 15 minutes. Remove the flasks from the water-bath, without drying them, and place in an incubator or another water-bath at 43°C.

Subculture the broth after 18-24 hours and again after 48 hours. Take one loopful of broth from the edge of the surface of the fluid and inoculate either two Brilliant Green Agar (Modified) CM329 plates (9cm diameter) without recharging the loop between plates, or one large plate (14cm diameter).

Incubate the plates at 35°C for 18-24 hours.

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

Precautions

Do not autoclave the base broth.
Add the iodine solution and brilliant green just prior to use.
The medium is not suitable for the growth of *S. typhi, S. sendai, S. pullorum* and *S. gallinarum.*

References

1 Muller L. (1923) *C.R. Soc. Biol. (Paris),* **89,** 434-443.
2 Kauffmann F. (1930) *Z.f. Hyg.*, **113,** 148-157.
3 Kauffmann F. (1935) *Z.f. Hyg.*, **117,** 26-32.
4 Jeffries L. (1959) *J. Clin. Path.*, **12,** 568-570.
5 Edel W. and Kampelmacher E.H. (1969) *Bull. Wld, Hlth. Org.*, **41,** 297-306.

Rappaport-Vassiliadis (RV) Enrichment Broth

Code: CM669

A selective enrichment broth for the isolation of salmonellae.

Formula (grams per litre)

Soya peptone	*5.0*
Sodium chloride	*8.0*
Potassium dihydrogen phosphate	*1.6*
Magnesium chloride. 6 H_2O	*40.0*
Malachite green	*0.04*
pH 5.2 ± 0.2	

THIS MEDIUM IS VERY HYGROSCOPIC AND MUST BE PROTECTED FROM MOISTURE

The quantities given for the formula as classically described made 1110ml of medium. They have been published this way in the Oxoid literature to coincide with the scientific literature.

The directions for reconstituting Oxoid Rappaport-Vassiliadis (RV) Enrichment Broth CM669 follow usual practice and specify the weight needed for 1 litre of medium.

Directions

Weigh 30g (the equivalent weight of dehydrated medium per litre) and add to 1 litre of distilled water. Heat gently until dissolved completely. Dispense 10ml volumes into screw-capped bottles or tubes and sterilize by autoclaving at 115°C for 15 minutes.

Description

Rappaport-Vassiliadis (RV) Enrichment Broth CM669 is based on the formulation described by van Schothorst and Renaud[1] and is recommended as the selective enrichment medium when isolating Salmonella from food and environmental specimens. It can also be used to isolate Salmonella from human faeces without pre-enrichment but the inoculum must be small. The original formulation described by Rappaport el al.[2] was specifically developed to exploit the four characteristics of Salmonella species when compared with other Enterobacteriaceae.

1 The ability to survive at relatively high osmotic pressures
2 To multiply at relatively low pH values.
3 To be relatively more resistant to malachite green.
4 To have relatively less demanding nutritional requirements.

Rappaport Broth was found[2] to be superior to Selenite Enrichment Broth and Tetrathionate Broth for enrichment of Salmonella with the exception of *S. typhi*. Vassiliadis et al.[3] modified Rappaport Broth by lowering the concentration of malachite green and raising the incubation temperature to 43°C. This modified Rappaport Enrichment Broth is RV or Rappaport-Vassiliadis Medium and has been found to be superior to other Salmonella selective enrichment media, especially when small inocula of pre-enrichment broth are used[4,5,6,7,8].

Oxoid Rappaport-Vassiliadis (RV) Enrichment Broth is similar to that described by Vassiliadis et al.[3] except that the peptone used is Soya Peptone, which has been reported to enhance the growth of Salmonella[1,9].

It is important that the inoculum size used for enrichment culture in RV Broth is sufficiently small not to interfere with its selectivity. Inoculum/broth ratios of 1:100 to 1:2000 have been suggested[10].

To improve the selectivity of RV Broth the addition of novobiocin was suggested[11].

Technique

Food and Environmental Specimens

1 Prepare Buffered Peptone Water (Oxoid CM509) as directed in containers containing 225ml of the medium.
2 Prepare Rappaport-Vassiliadis (RV) Enrichment Broth CM669 as directed.
3 Add 25g of the test specimen to 225ml of Buffered Peptone Water and incubate at 35°C for 16-20 hours.
4 Inoculate 0.1ml of the pre-enrichment peptone water culture to 10ml of Rappaport-Vassiliadis (RV) Enrichment Broth and incubate at 42°C ± 1°C for 24-48 hours.*
5 Subculture the broth by streaking on to plates of Brilliant Green Agar (Modified) CM329. Incubate at 35°C for 18-24 hours.
6 Colonies showing typical *Salmonella* colonial morphology should be confirmed by biochemical or serological methods.

*The recommended incubation temperature is 43°C but this is a critical upper limit. To allow for incubator temperature fluctuation 42°C ± 1°C is a preferred recommendation with 42°C ± 0.1°C for water baths. Preheat the enrichment broth to 43°C before inoculation.

Faecal Specimens Without Pre-enrichment

Add one or two 3mm loopsful of liquid faeces (or an emulsion of faeces in saline) to 10ml of RV Broth CM669 pre-heated to 43°C. Incubate at 42°C ± 1°C for 24-48 hours.

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

Precautions

RV Broth should not be used if *Salm.typhi* is suspected.
Note the difference in weight between the classical formula on the label and the reduced weight per litre, using anhydrous magnesium chloride.

References

1 van Schothorst M. and Renaud A.M. (1983) *J. Appl.Bact.*, **54,** 209-215.
2 Rappaport F., Konforti N. and Navon B. (1956) *J. Clin. Path.*, **9,** 261-266.
3 Vassiliadis P., Pateraki E., Papiconomou N., Papadakis J.A. and Trichopoulos D. (1976a) *Annales de Microbiologie (Institut Pasteur)*, **127B,** 195-200.
4 Vassiliadis P., Trichopoulos D., Kalapothaki V. and Serie C. (1981) *J. Hyg. Camb.*, **87,** 35-39.
5 Harvey R.W.S., Price T.H. and Xirouchaki E. (1979) *J. Hyg. Camb.*, **82,** 451-460.
6 Vassiliadis P. (1983) *J. Appl. Bact.*, **54,** 69-75.
7 Vassiliadis P., Kalapothaki V., Trichopoulos D., Mavromatte C. and Serie C. (1981) *Appl. & Environ. Microbiol.*, **42,** 615-618.
8 Vassiliadis P. (1983) *J. Appl. Bact.*, **56,** 69-76.
9 McGibbon L., Quail E. and Fricker C.R. (1984) *Inter. J. Food Microbiol.*, **1,** 171-177.
10 Fricker C.R. (1987) *J. Appl. Bact.*, **63,** 99-116.
11 Fricker C.R. (1984) *J. Appl. Bact.*, **56,** 305-309.

Rappaport-Vassiliadis Soya Peptone (RVS) Broth

Code: CM866

A selective enrichment broth for the isolation of salmonellae.

Formula (grams per litre)

Soya peptone	*4.5*
Sodium chloride	*7.2*
Potassium dihydrogen phosphate	*1.26*
Di-potassium hydrogen phosphate	*0.18*
Magnesium chloride (anhydrous)	*13.58*
Malachite green	*0.036*

pH 5.2 ± 0.2

Directions

Suspend 26.75 grams in 1 litre of distilled water and heat gently to dissolve. Dispense 10ml volumes into screw-capped bottles or tubes and sterilize by autoclaving at 115°C for 15 minutes.

Description

Rappaport-Vassiliadis Soya Peptone (RVS) Broth is recommended as a selective enrichment medium for the isolation of Salmonellae from food and environment specimens.

RVS Broth CM866 shares with the original formulation[2], the ability to exploit the full characteristics of *Salmonella* species when compared with other Enterobacteriaceae. These are:

1 The ability to survive at relatively high osmotic pressure.
2 To multiply at relatively low pH values.
3 To be relatively more resistant to malachite green.
4 To have relatively less demanding nutritional requirements. The use of di-potassium hydrogen phosphate ensures that the pH of the prepared medium is maintained during storage.

RVS broth is based on the revised formulation described by van Schothorst et al[1], and is recommended as the selective enrichment medium for the isolation of salmonellae from food and environmental specimens. It can also be used to isolate salmonellae from human faeces without the need for pre-enrichment.

RVS Broth is a modification of the Rappaport Vassiliadis (R10) Enrichment Broth described earlier by van Schothorst and Renaud[3]. The modifications to their earlier formula are:

1 The addition of di-potassium hydrogen phosphate to buffer the medium so that the pH is maintained during storage of the prepared broth.
2 Clarifying the optimum concentration of magnesium chloride $6H_2O$.

The two modifications are said to enhance the reliability of the enrichment broth[1]. Peterz et al[4] have also highlighted the importance of the concentration of magnesium chloride in the final medium.

Technique

Food and Environmental Specimens

1 Prepare Buffered Peptone Water (Oxoid CM509) as instructed on the label in volumes of 225ml.
2 Prepare RVS Broth CM866 as instructed.
3 Add 25g or 25ml of the test sample to 225ml of Buffered Peptone Water and incubate at 37°C for 16-20 hours. Transfer 0.1ml of the pre-enrichment peptone water culture to 10ml of RVS Broth and incubate at 42°±1.0°C for 24 hours.
4 Subculture the enrichment broth by streaking onto plates of MLCB Agar CM783 and Brilliant Green Agar (Modified) CM329. Incubate at 35°C for 18-24 hours. Colonies suspected as salmonellae should be confirmed by biochemical or serological methods.

Faecal specimens – no pre-enrichment needed. Add one or two 3mm loopsfull of liquid faeces (or an emulsion of faeces in saline) to 10ml of RVS Broth CM866 pre-warmed to 42°C. Incubate at 42°C±1.0°C for 24 hours, and then streak onto selective agars of choice.

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

Precautions

RVS Broth should not be used if *Salm.typhi* is suspected.
In order to achieve optimum recovery it is recommended that the enrichment broth is incubated at 42°C±0.1°C.

References

1 van Schothorst M., Renaud A. and van Beek C. (1987) *Food Microbiology*, **4,** 11-18.
2 Rappaport F., Konforti N. and Navon B. (1956) *J. Clin. Pathol.*, **9,** 261.
3 van Schothorst M. and Renauld A. (1983) *J. Appl. Bact.*, **54,** 209-215.
4 Peterz M., Wiberg C. and Norberg P. (1989) *J. Apol. Bact.*, **66,** 523-528.

Modified Semi-Solid Rappaport-Vassiliadis (MSRV) Medium

MSRV Medium Base **Code:** CM910 **MSRV Selective Supplement** SR161

A semi-solid medium for the detection of motile Salmonella species from food and enrivonmental samples.

MSRV Medium Base

Formula (grams per litre)

MSRV Medium Base (grams per litre)	
Tryptose	*4.59*
Casein hydrolysate	*4.59*
Sodium chloride	*7.34*
Potassium dihydrogen phosphate	*1.47*
Magnesium chloride (anhydrous)	*10.93*
Malachite green oxalate	*0.037*
Agar	*2.7*
Final pH 5.2 ± 0.2	

This medium is very hygroscopic and must be protected from moisture.

MSRV Selective Supplement

Vial Contents

Novobiocin	*10mg*

Each vial is sufficient to supplement 500ml of MSRV medium base.

Directions

Suspend 15.8g of MSRV Medium Base in 500ml of distilled water. Bring to the boil with frequent agitation.

DO NOT AUTOCLAVE.

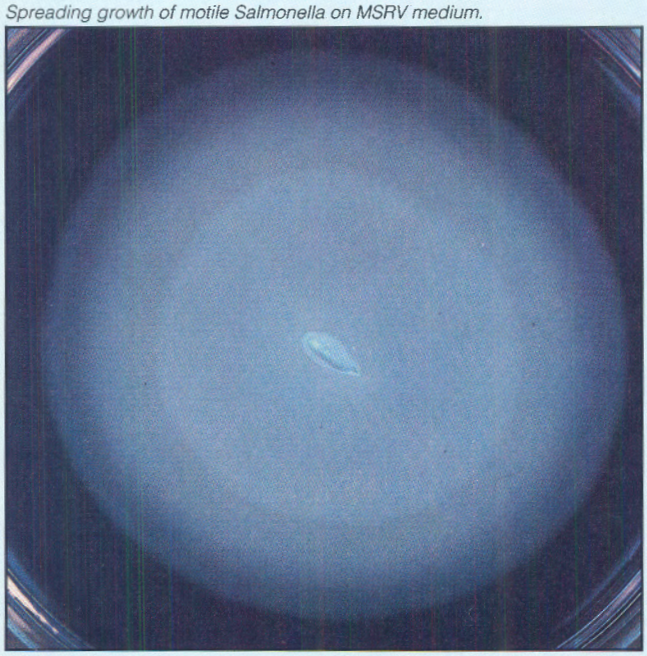
Cool to 50°C and aseptically add the contents of 1 vial of MSRV Selective Supplement reconstituted with 2ml of sterile distilled water. Mix well and pour into sterile petri dishes. Air dry at room temperature for at least one hour. (Plates may be air-dried overnight prior to storage at 2°C to 8°C).

Description

A semi-solid medium for the detection of motile Salmonella spp. from food and environmental samples.

Modified Semi-Solid Rappaport Vassiliadis (MRVS) medium is based on the formation described by De Smedt et al which has been shown to detect more Salmonella-positive samples than the traditional enrichment procedures[1,2]. Further collaborative studies have confirmed these findings[3,4].

Spreading growth of motile Salmonella on MSRV medium.



Motility enrichment on MSRV Medium has been designed as a simple, sensitive method for the isolation of Salmonella from food and environmental samples. The efficiency of the medium is based on the ability of Salmonella to migrate through the semi-solid selective medium ahead of competing motile organisms, thus producing opaque halos of growth.

Further tests can be carried out directly from the migrated culture with the inoculum being taken from the edge of the growth.

The medium is not suitable for the detection of non-motile strains of Salmonella (incidence <0.1%)[5]. (Figures obtained from records of the Department of Enteric Pathogens, Central Public Health Laboratory, Colindale, London. Dr. B. Rowe, Personal Communication. 1988).

If the presence of non-motile Salmonella is suspected the pre-enrichment culture should also be plated onto a selective agar medium.

Technique

1 Inoculate three drops (ca. 0.1ml) of the pre-enrichment culture (after incubation for 16-20 hours) in separate spots on the surface of the MSRV medium plates.
2 Incubate the plates in an upright position at 42°C for up to 24 hours. (Care should be taken not to exceed 24 hours).
3 Examine the plates for motile bacteria which will be shown by a halo of growth originating from the inoculation spot.
4 Sub-cultures can be taken from the outside edge of the halo to confirm purity and for further biochemical and serological tests.

Storage conditions and Shelf life

MSRV Medium Base should be stored tightly capped in the original container in a cool, dry place away from bright light. When stored as directed the medium will remain stable until the expiry date printed on the label.

MSRV Selective Supplement should be stored in the dark at 2°C to 8°C. When stored as directed before rehydration, the antibiotic remains stable until the expiry date printed on the label.

The prepared plates may be stored for up to 14 days at 2°C to 8°C in the dark.

Quality Control

Salmonella typhimurium	ATCC 14028	Straw colonies at site of inoculation surrounded by halo of growth.
Salmonella enteritidis	ATCC 13076	Straw colonies at site of inoculation surrounded by halo of growth.
Citrobacter freundii	ATCC 8090	Restricted or no growth.

Precautions

MSRV should not be used if *Salm.typhi* is suspected.

References

1 De Smedt, J.M., Bolderdijk, R., Rappold, H. and Lautenschlaeger, D. (1986) *J. Food. Prot.*, **49,** 510-514.
2 De Smedt, J.M., Bolderdijk, R., (1987) *J. Food. Prot.*, **50,** 658-661.
3 De Zutter, L. et al., (1991) *Int. J. Food Micro.*, **13,** 11-20.
4 De Smedt, J.M. et al., (1991) *Int. J. Food Micro.*, **13,** 301-308.
5 Holbrook, R., Anderson, J.M., Baird-Parker, A.C., Dodds, L.M., Sawhney, D., Struchbury, S.H. and Swaine, D. (1989) *Lett. Appl. Microbiol.*, **8,** 139-142.

Plating Media

Plating media used in the culture of Salmonella contain selective agents generally similar to those employed in Salmonella selective enrichment broths. Bile salts and brilliant green are in common use. A diagnostic system is also incorporated to permit differentiation of Salmonella from accompanying flora enabling suspect colonies to be recognised more easily so that they may be picked for further testing. The selective agents and diagnostic systems used in various media formulae are listed in Table 2.

TABLE 2 — Selective agents and presumptive identification systems used in plating media for the isolation of Salmonella.

Medium	Selective Agent	Identification System
Brilliant Green Agar	Brilliant green. Sulphonamides-optional. Sodium sulphacetamide and sodium mandelate – optional	Lactose and sucrose fermentation.
MLCB Agar	Brilliant green Crystal violet	Hydrogen sulphide (H_2S) production.
XLD Agar	Deoxycholate	Lactose, sucrose, xylose fermentation. H_2S production. Lysine decarboxylation.
Bismuth Sulphite Agar	Bismuth sulphite Brilliant green	Sulphite – sulphide reduction in presence of fermentable carbohydrate.
Hektoen Agar	Bile salts Novobiocin-optional	Lactose, sucrose & salicin fermentation. H_2S production.
Desoxycholate-Citrate Agar	Deoxycholate Sodium citrate Sodium thiosulphate	Lactose fermentation. H_2S production.
SS Agar	Brilliant green bile salts	Lactose fermentation. H_2S production.
DCLS Agar	Deoxycholate Sodium citrate Sodium thiosulphate	Lactose & sucrose fermentation. H_2S production.

Not all serovars of Salmonella will grow equally well on all the plating media and the success with which the media suppress contaminating flora also differs. Recovery of the widest possible range of Salmonella serovars requires two or more plating media. Actual choice, where this is not strictly laid down by specific protocol, should take account of the food under investigation and the competing organisms that are likely to be present with the Salmonella in the selective enrichment broth. Many plating media are inhibitory towards *S. typhi* and where presence of this organism is a possibility, Bismuth Sulphite agar should certainly be used. Bismuth Sulphite agar is also useful for detection of lactose fermenting Salmonella serovars. Many plating media designed for the presumptive identification of Salmonella employ as a principle the usual inability of the organism to ferment lactose. However, lactose fermenting strains occur and unless their presence is suspected they may be discarded. The problem of misidentification when reading plates is recognised in many formulae by the incorporation of additional diagnostic tests in order to make detection more certain. Media may contain lactose and one of a number of other fermentable carbohydrates e.g. sucrose, salicin and xylose. Enterobacteriaceae that are not Salmonella will ferment at least one of these additional carbohydrates, greatly reducing the number of non-fermenting colonies that are present and therefore the need to investigate further. Media containing bile salt as an inhibitor generally have a hydrogen sulphide indicator system. Salmonella colonies appear as non-fermenting "bulls-eye" colonies with a black centre. This appearance is also commonly shown by Proteus species and may be particularly troublesome if a medium contains lactose as the only fermentable carbohydrate.

Lysine decarboxylation by Salmonella is also employed as a diagnostic feature and combination of this with other tests such as carbohydrate fermentation and H_2S production offers microbiologists the opportunity to identify presumptively a wide range of Enterobacteriaceae by the characteristic appearances they exhibit. This serves to eliminate them from the need for further investigation.

None of the Salmonella plating media are fully selective. Choice of medium has to take account of other organisms that are likely to be present. Brilliant Green agar is effective for investigating many foods but may permit the growth of Pseudomonas and Proteus species. This undesirable growth may be controlled by the incorporation of sodium sulphacetamide and mandelic acid (Sulphamandelate Supplement Code SR87) in the medium but some inhibition of Salmonella may result.

The range of flora able to grow on MLCB agar is similar to that for Brilliant Green agar. The strikingly characteristic appearance shown by Salmonella on MLCB agar as a result of lysine decarboxylation and H_2S production permits the organism to be differentiated easily from most of the accompanying flora.

Hektoen agar is less selective than Brilliant Green agar but typical non-lactose fermenting, H_2S positive colonies of Salmonella are readily recognised by their appearance even amongst a growth of competing organisms. Additional selectivity may be conferred by adding novobiocin to the medium.

The foregoing examples should serve to illustrate considerations that should be applied to selection of appropriate media for plating of Salmonella in selective enrichment culture. A summary of the relative selectivity and usefulness of the Oxoid media described in the following pages is given in Table 3.

TABLE 3 — Advantages and limitations of broth and plating media for Salmonella species.

Pre-Enrichment Broths	**Advantages**	**Limitations**
Buffered peptone water	High pH buffering capacity	Overgrowth with Gram-positive and Gram-negative bacteria. Optional control with brilliant green[1], crystal violet[1], malachite green[2].
Lactose broth		Contains fermentable carbohydrates. Lacks pH buffering capacity.
Lab-Lemco broth		
Tryptone soya broth		
Selective Enrichment Broths		
Selenite F	Will grow a wide range of sevovars including *S. typhi.*	Not very selective.
Selenite cystine broth	Cystine enhances Salmonella growth. Inoculum to enrichment broth ratio not critical.	Not very selective.
Mannitol selenite broth	Enhances *S. typhi* growth.	Not very selective.
Tetrathionate broth (USP)	Use instead of Muller-Kauffmann Tetrathionate Broth for *S. typhi.*	
Muller-Kauffmann tetrathionate broth	Improved selectivity. Inoculum to enrichment broth ratio not critical.	Inhibitory to *S. typhi*, S. *pullorum* and S. *gallinarum.*
Tetrathionate broth (Oxoid CM29)	Will grow a wide range of serovars including *S. typhi.*	This medium does not appear amongst the enrichment media listed by ISO, APHA, AOAC and IDF.
Rappaport (RV) broth	Superior productivity to selenite and tetrathionate broths for most serovars.	Inhibitory to *S. typhi* and some other serovars. Inoculum to enrichment broth ratio critical. pH of broth may fall on storage.
Rappaport (RVS) broth	More productive than Rappaport (RV) broth. pH of medium does not reduce on storage.	Inhibitory to *S. typhi* and some other serovars. Inoculum to enrichment broth ratio critical.
Selective Agar Media		
Bismuth sulphite agar	Will detect *S. typhi* and lactose – fermenting Salmonella.	Variable performance.
Brilliant green agar	Commonly used in the food industry.	Not suitable for *S. typhi.*
Hektoen agar	Good differentiation of typical Salmonella strains. May also be used for Shigella.	Not very selective.
XLD agar	Good differentiation of typical Salmonella strains. May also be used for Shigella.	Not very selective.
MLCB agar	Good differentiation of typical Salmonella strains. Particularly useful for detecting lactose-fermenting strains.	Not very selective. Not suitable for H_2S-negative strains or *S. typhi.*
Selective Agar Media		
Desoxycholate-citrate agar	May also be used for Shigella.	Not very selective.
S.S. agar	More selective than desoxycholate-citrate agar. May also be used for Shigella.	

References
1 North, W.R. *J. Bact.*, (1960), **80,** 861.
2 van Schothorst, M. and Renaud, A. (1983) *J. Appl. Bact.*, **54,** 209-215.

Bismuth Sulphite Agar

Code: CM201

A modification of the original Wilson and Blair Medium for the isolation of *Salmonella typhi* and other salmonellae. It is particularly useful for the isolation of lactose-fermenting salmonellae.

Formula (grams per litre)

Peptone	*5.0*
'Lab-Lemco' powder	*5.0*
Glucose	*5.0*
Disodium phosphate	*4.0*
Ferrous sulphate	*0.3*
Bismuth sulphite indicator	*8.0*
Brilliant green	*0.016*
Agar	*12.7*
pH 7.6 ± 0.2	

Directions (half litre volume)
Suspend 20g in 500ml of distilled water in a 1 litre flask. Heat gently with frequent agitation until the medium just begins to boil and simmer for 30 seconds to dissolve the agar. Cool to 50°-55°C, mix well to disperse the suspension and pour thick plates (25ml medium per plate). Allow the medium to solidify with the dish uncovered. Larger volumes may be prepared if great care is taken and adequate head space provided.

Dry the plates before use but take care to avoid overdrying. Correctly prepared plates should have a smooth, cream-like opacity with a pale straw colour. There should be no sedimentation of the indicator.

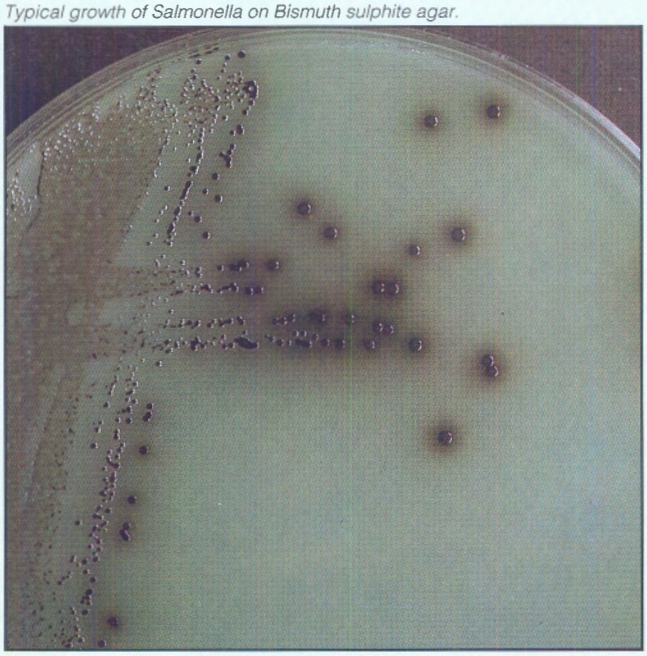
DO NOT OVERHEAT – DO NOT AUTOCLAVE.

Description
Bismuth Sulphite Agar is a modification of the original Wilson and Blair[1] selective medium for the isolation and preliminary identification of *Salmonella typhi* and other salmonellae from pathological material, sewage, water supplies, food and other products suspected of containing these pathogens.

In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of salmonellae. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide.

Atypical colonies may appear if the medium is heavily inoculated with organic matter. Such a situation may be prevented by suspending the sample in sterile saline and using the supernatant for inoculation.

Typical growth of Salmonella on Bismuth sulphite agar.



The freshly prepared medium has a strong inhibitory action[2] and is suitable for heavily contaminated samples. Storing the poured plates at 4°C for 3 days causes the medium to change colour to green, making it less selective with small numbers of salmonellae being recovered[3]. However, for *Salmonella typhi* recovery the latter technique is not recommended[4]

Where the number of salmonellae is expected to be small, enrichment methods may be employed.

The use of this medium is advocated by several authorities[5,6,7].

Technique
Bismuth Sulphite Agar may be used in conjunction with other selective enteric agars for the isolation of salmonellae by direct plating or from enrichment media[8]. Thus the following scheme may be adopted.

Inoculate directly on Bismuth Sulphite Agar and one or more of the following:

Desoxycholate Citrate Agar CM227 *or* DCLS Agar CM393
XLD Agar CM469
Brilliant Green Agar CM329
MacConkey Agar No.3 CM115

At the same time inoculate an enrichment broth, such as Selenite Broth Base CM395 + Sodium Biselenite L121 or Tetrathionate Broth CM343. Subculture on to Bismuth Sulphite Agar and any other selective medium after 12-18 hours incubation. Examine the plates after 18 hours incubation and subculture suspect colonies to identification media, e.g. Kligler Iron Agar CM33.

All negative plates should be incubated for 48 hours.

Salmonella typhi
Appearance
Black 'rabbit-eye' colonies with a black zone and metallic sheen surrounding the colony after 18 hours. Uniformly black after 48 hours incubation.

Other Salmonella species
Appearance
Variable colony appearance after 18 hours, they may be black, green or clear and mucoid. Uniformly black colonies are seen after 48 hours, often with widespread staining of the medium and a pronounced metallic sheen.

Other organisms, e.g. coliform bacteria, *Serratia, Proteus* species
Appearance
Usually inhibited but occasional strains give dull green or brown colonies with no metallic sheen or staining of the surrounding medium.

Storage conditions and Shelf life
Store the dehydrated medium below 25°C and use before the expiry date on the label. Note the following comments:

Due to its contents of reactive and hygroscopic substances, dehydrated Bismuth Sulphite Agar quickly deteriorates when exposed to the atmosphere. This is usually indicated by aggregation into a solid non-friable mass, and by the development of a brown coloration. Medium reconstituted from such material is brown, does not become green on storage, and is characterized by loss of differential and selective properties. For this reason the powder should be stored in a cool, dry place and after use the container should be properly closed.

Prepared medium
It is recommended that the medium should be used on the day of preparation.

Quality Control
Salmonella typhi should be used only in a Class II laboratory. Not for routine testing or in food laboratories.

Positive control:
Salmonella enteritidis ATCC® 13076
S. typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Citrobacter freundii ATCC® 8090

Precautions
Prepared plates of medium should not be stored for longer than two days at 2-8°C; after which time the dye oxidizes to give a green medium that can be inhibitory to some salmonellae.

Shigella species are usually completely inhibited.

Salmonella sendai, S. cholera-suis, S. berta, S. gallinarum and *S.abortus-equi* are markedly inhibited[9].

It is important that the spreading technique yields well separated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S. typhi* colonies will appear light green in these circumstances. Therefore, when in doubt, almost any growth on the medium should be subject to further tests.

References

1 Wilson W.J. and Blair E.M.McV (1927) *J. Hyg. Camb.*, **26,** 374.
2 Cook G.T. (1952) *J. Path. Bati.*, **64,** 559.
3 McCoy J.M. and Spain G.E. (1969) *in Isolation Methods for Microbiologists, p.20. Ed. by Shapton D.A. and Gould G.W., Academic Press London.*
4 Hobbs B.C., King G.C.G. and Allison V.D. (1945) *Monthly Bulletin of the Ministry of Health and Emergency Public Health Lab. Service* **4,** 40.
5 Anon (1981) *Int. Standard ISO 6579-1981. Geneva. Internat. Organisation for Standardization.*
6 ICMSF (1978) *Micro-organisms in Food 1. 2nd Edn. University of Toronto Press, Ontario.*
7 Speck M.L. (1984) *Compendium of methods for the microbiological examination of foods. 2nd Edn. American Public Health Association.*
8 Harvey R.W.S. and Price T.M. (1974) *Public Health Laboratory Service Monograph Series No.8. Isolation of Salmonellas. HMSO London.*
9 Hajna A.A. (1951) *Pub. Hlth. Rep.*, **9,** 48-51.

Brilliant Green Agar

Code: CM263

A selective medium for the isolation of salmonellae, other than *S. typhi.*

Formula (grams per litre)

Proteose peptone	*10.0*
Yeast extract	*3.0*
Lactose	*10.0*
Sucrose	*10.0*
Sodium chloride	*5.0*
Phenol red	*0.08*
Brilliant green	*0.0125*
Agar	*12.0*
pH 6.9 ± 0.2	

Directions (half litre volume)
Suspend 50g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

Description
Brilliant Green Agar was first described as a selective isolation medium for *Salmonella* species by Kristensen et al.[1] Kauffmann[2] modified their formula to give a highly selective plating medium for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products. This medium was not designed for the isolation of *Salmonella typhi* or *Shigella* species and where these may be encountered, Brilliant Green Agar should be used in parallel with other selective plating media such as Desoxycholate Citrate Agar (Hynes) CM227, Hektoen Enteric Agar CM419, XLD Agar CM469. Bismuth Sulphite Agar (Modified) CM201 is specifically recommended for *Salmonella typhi.*

The use of enrichment/selective broths prior to subculture on Brilliant Green Agar will improve the probability of isolating salmonellae. Tetrathionate Broth Base CM29, Tetrathionate Broth USA. CM671, Selenite Broth Base CM395 and Muller Kauffmann Tetrathionate Broth Base CM343 may be used in conjunction with Brilliant Green Agar.

Brilliant Green Agar corresponds to the medium recommended by the APHA[3,4] and the AOAC[5].

The addition of sulphonamides to Brilliant Green Agar helps improve the isolation of salmonellae[6]. To one litre of Brilliant Green Agar add 1.0g of sulphapyridine or 0.8g sulphadiazine and sterilize in the normal way.

Typical growth of Salmonella on Brilliant green agar.

Technique

Examination of faeces, or similar material, for salmonellae:

1 Heavily inoculate a Brilliant Green Agar plate. At the same time, inoculate other plating media and tubes of Selenite Broth and Tetrathionate Broth.
2 Incubate the Brilliant Green Agar plate for 18-24 hours at 35°C.
3 Examine the plates and identify suspect colonies using differential tests for serological methods.
4 If no non-lactose fermenters are observed on the primary plate cultures, inoculate Brilliant Green Agar and other medium with the enrichment cultures – then proceed as in paragraph 3.

Examination of Foods

1 Pre-enrich four 25g aliquots of food in 75ml of Buffered Peptone Water CM509 and incubate at 35°C for 4-6 hours.
2 Add to each sample 75ml of double-strength Selenite Cystine Broth CM699 and incubate at 43°C for 24 hours.
3 Subculture to plates of Brilliant Green Agar and Bismuth Sulphite Agar (Modified) CM201.
4 Incubate the plates at 35°C and examine the Brilliant Green Agar after 24 hours and the Bismuth Sulphite Agar after 48 hours.
5 Look for colonies with salmonella characteristics and confirm their identity with biochemical and serological tests.

Examination of food for salmonellae (enumeration)[4]
This is carried out by adding equal volumes of decimal dilutions of the homogenized sample to tubes of double strength Selenite Broth. After incubation, a loopful from each tube is plated on Bismuth Sulphite Agar and Brilliant Green Agar. Colonies with salmonellae characteristics are identified and the most probable number of salmonellae per gram of sample is calculated from the three highest sample dilutions which yield salmonellae on subculture.

Examination of dairy products for salmonellae[3]
Milk and liquid milk products, dried milk, cheese, eggs and egg products – Brilliant Green Agar is employed, with and without an enrichment phase, in conjunction with other selective media for enteric bacteria.

Colonial Characteristics

Non-lactose/sucrose fermenting organisms
Red-pink-white opaque coloured colonies surrounded by brilliant red zones in the agar – most probably salmonella (but not *S. typhi*).

Proteus and Pseudomonas species
These may grow as small red colonies.

Lactose/sucrose fermenting organisms (normally inhibited)
Yellow to greenish-yellow coloured colonies surrounded by intense yellow-green zones in the agar – E. coli or *Klebsiella/Enterobacter* group.

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 of medium at 2-8°C.

Quality Control
Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Proteus vulgaris ATCC® 13315

Precautions

Lactose-fermenting salmonella (*S. arizona*) may be present in foods[7].

Salmonella typhi and *Shigella* species may not grow on this medium. Use the cited alternative media.

Proteus, Citrobacter and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

References

1 Kristensen M., Lester V. and Jurgens A. (1925) *Brit. J. Exp. Pathol.*, **6,** 291-297.

2 Kauffmann F. (1935) *Seit. F. Hyg.*, **177,** 26-34.

3 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington D.C.*

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

5 Association of Official Analytical Chemists (1978) *Bacteriological Analytical Manual 5th Edn. AOAC. Washington D.C.*

6 Osborn W. W. and Stokes J.L. (1955) *Appl. Microbiol.*, **3,** 295-301.

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Brilliant Green Agar (Modified)

Code: CM329

A selective and diagnostic agar for salmonellae (other than *S. typhi*) from food and feeds.

Formula (grams per litre)

'Lab-Lemco' powder	*5.0*
Peptone	*10.0*
Yeast extract	*3.0*
Disodium hydrogen phosphate	*1.0*
Sodium dihydrogen phosphate	*0.6*
Lactose	*10.0*
Sucrose	*10.0*
Phenol red	*0.09*
Brilliant green	*0.7*
Agar	*12.0*
pH 6.9 ± 0.2	

Directions

Suspend 52 grams in 1 litre of distilled water. Heat gently with occasional agitation and bring just to the boil to dissolve the medium completely.

DO NOT AUTOCLAVE.

Cool to 50°C, mix well and pour plates.

Sulphamandelate Supplement
Code: SR87

Vial contents (each vial is sufficient for 500ml of medium)

Sodium sulphacetamide	500mg
Sodium mandelate	125mg

Directions

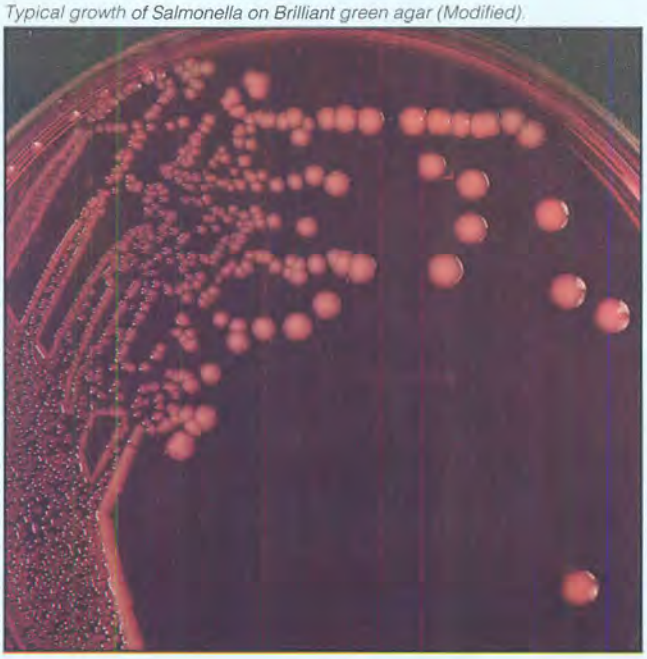
To one vial add 5ml of sterile distilled water and mix gently to dissolve the contents completely. Avoid frothing. Add the solution to 500ml of sterile Oxoid Brilliant Green Agar (Modified) CM329 cooled to 50-55°C. Mix gently and pour into sterile petri dishes.

Description

Brilliant Green Agar (Modified) was developed from a formula supplied by the Rijks Instituut voor de Volksgezondheid (National Institute for Public Health), Utrecht[1,2].

The medium has been widely assessed in Europe and is now used in the ISO standards[3,4,5].

Typical growth of Salmonella on Brilliant green agar (Modified).



The advantages claimed for the medium are the greater inhibition of *Escherichia coli* and *Proteus* species than other formulations: the restriction of growth of *Pseudomonas* species, whose colonies may resemble salmonellae on Brilliant Green Agar and cause confusion or much extra work to confirm their identity: the absence of inhibitory properties towards small numbers of salmonellae[6].

Selective Brilliant Green Agar (Modified)

Watson and Walker[7] incorporated a combination of sulphacetamide (at 1.0 mg/ml) and mandelic acid (at 0.25 mg/ml) into Oxoid Brilliant Green Agar (Modified) to obtain maximum recovery of salmonellae from Muller Kauffmann Tetrathionate Broth whilst giving maximum suppression of contaminating organisms.

Oxoid Salmonella Sulpha-Mandelate Supplement, SR87 used for the isolation and enumeration of salmonellae from sewage and sewage sludge, is based on the formulation of Watson and Walker[1,7]. These authors showed that the use of Brilliant Green Agar (Modified) CM329 incorporating a combination of sulphacetamide (1.0 mg/ml) and mandelic acid (0.25 mg/ml) incubated at 43°C resulted in maximum recovery of salmonellae from Muller-Kauffman Tetrathionate Broth.

The method described[7] has been shown to be a quick and reliable technique for the isolation of sub-lethally damaged salmonellae from treated sewage and sewage sludge.

Use of antibiotic supplemented Brilliant Green Agar is made necessary because the pre-enrichment of the sewage in phosphate buffered peptone (PBP) water will encourage not only the growth of stressed salmonellae but many competing organisms.

The inhibitory properties of Muller-Kauffman Tetrathionate Broth are not sufficient by themselves to suppress the growth of the latter. The advantage claimed for Selective Brilliant Agar is its greater inhibition of contaminating organisms and a lower incidence of false positives.

This advantage was confirmed by Fricker and his co-workers when using Brilliant Green Agar (Modified) CM329 containing sodium sulphacetamide and sodium mandelate for plating enrichment cultures in Rappaport Broth, from sewage and sewage polluted water[8,11] seagull faeces[9] and chicken[10,12].

Vassiliadis et al.[13] added 2.5g of sodium desoxycholate L57 to one litre of Brilliant Green Agar (Modified) to prevent swarming by *Proteus hauseri*, during examination of sewage effluents. They found desoxycholate to be superior to sulphonamides in suppressing swarming without affecting the growth of a wide range of salmonella serotypes.

Colonial Characteristics

Salmonellae – red colonies surrounded by bright red medium.

Lactose/Sucrose fermenters – inhibited to a certain extent, but producing yellow green colonies when growth is evident.

Proteus – almost completely inhibited, those colonies that grow produce red colonies without swarming.

Pseudomonas – inhibited growth of small, crenated red colonies.

Technique

Technique for food and feeds

An outline of the method used by Edel and Kampelmacher[2] in their trials is as follows:

1 One part of the food sample was added to 20 parts of Muller Kauffmann Tetrathionate Medium CM343.
2 After agitation, the flask of broth was placed into a 45°C waterbath for 15 minutes only.
3 The flask was then transferred to a 43°C incubator.
4 The broth was subcultured to Brilliant Green Agar (Modified) after 18 and 48 hours.
A single loopful of broth was used to streak inoculate either two 9cm diameter plates (without recharging the loop between plates) or one 14cm diameter plate.
5 The plates were incubated at 35°C for 18-24 hours.
6 Red colonies, resembling salmonellae, were picked off the plates and subcultured to Lysine Decarboxylase Broth CM308 and Triple Sugar Iron Agar CM277. These media were incubated at 35°C for 18-24 hours.

If the reactions on these media were positive for salmonellae then slide agglutination tests were carried out on the surface growth of the Triple Sugar Iron Agar.

Technique for sewage[7]

1 Take a representative sample of sewage or sludge for examination.
2 Homogenise a suitable volume in a macerator or stomacher.
3 Inoculate five 10ml samples into 35ml of Buffered Peptone Water CM509, five 1ml samples and five 0.1ml samples into 10ml of Buffered Peptone Water. Incubate at 35°C overnight.
4 Transfer 10ml portions into 35ml of Muller Kauffmann Tetrathionate Broth and incubate at 43°C.
5 Subculture the broths on to Brilliant Green Agar (Modified) containing Sulphamandelate Selective Supplement SR87 after 24 and 48 hours incubation.
6 Incubate the Brilliant Green Agar plates overnight at 43°C.
7 Identify suspicious (red) colonies using further identification tests.

The Sulphamandelate Selective Supplement SR87 inhibits competing organisms which multiply during the resuscitation and recovery stages in Buffered Peptone Water.

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 of medium at 2-8°C.

Quality Control

Positive control:
Salmonella typhimurium ATCC® 14028

Negative control:
Escherichia coli ATCC® 25922
Proteus vulgaris ATCC® 13315

Precautions

Lactose-fermenting salmonellae may be present in foods[14].

Salmonella typhi and *Shigella* species may not grow on this medium.

Proteus, Citrobacter and *Pseudomonas* species may mimic enteric pathogens by producing small red colonies.

References

1 Edel W. and Kampelmacher E.H. (1968) *Bull. Wld. Hlth. Org.*, **39,** 487-491.
2 Edel W. and Kampelmacher E.H. (1969) *Bull. Wld. Hlth. Org.*, **41,** 297-306.
3 Anon. (1975) *International Organisation for Standardization. Meat and Meat products* – detection of Salmonella. Ref. method ISO 3565-1975(E).
4 Anon. (1981) *International Organization for Standardization. Microbiology* – General guidance on methods for the detection of Salmonella. Ref. method ISO 6579-1981(E).
5 Anon. (1985) *International Organization for Standardization. Milk and Milk products* – detection of Salmonella. Ref. method ISO 6785-1985.
6 Read R.B. and Reyes A.L. (1968) *Appl. Microbiol.*, **16,** 746-748.
7 Watson U.C. and Walker A.P. (1978) *J. Appl. Bact.*, **45,** 195-204.
8 Fricker C.R. (1984) *Zbl. Bakt. Hyg. Abt. I. Orig. B.*, **179,** 170-178.
9 Fricker C.R. and Girdwood R.W.A. (1984) *J. Hyg.*, **93,** 35-42.
10 Fricker C.R. (1984) *Int. J. Food Microbiol*, **1,** 171-177.
11 Fricker C.R. and Girdwood R.W.A. (1985) *J. Appl. Bact.*, **58,** 343-346.
12 Fricker C.R., Quail E., McGibbon L. and Girdwood R.W.A. (1985) *J. Hyg.*, **95,** 337-344.
13 Vassilliadis P., Trichopoulos J., Papadakis V.K. and Ch. Serie. (1979) *Ann. Soc. belge. Med. trop.*, **59,** 117-120.
14 Harvey R.W.S., Price T.H. and Hall L.M. (1973) *J. Hyg. Camb.*, **71,** 481-486.

DCLS Agar

Code: CM393

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

Formula (grams per 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 50g 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

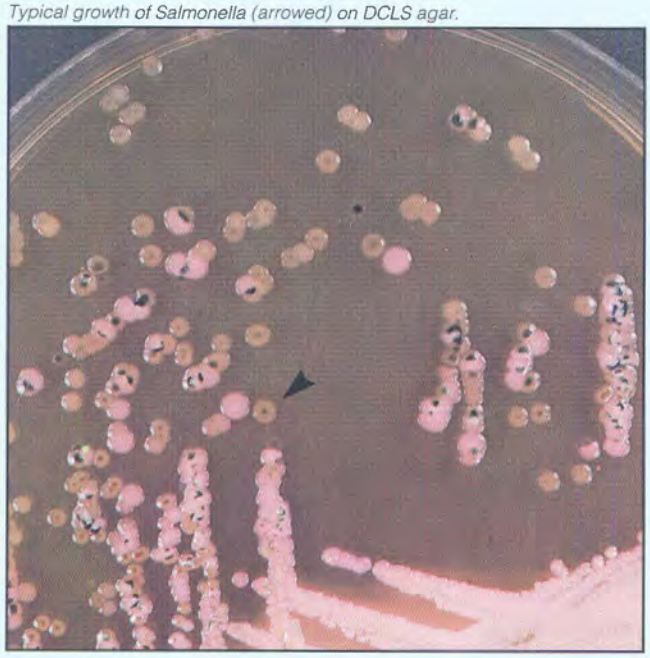
DCLS Agar is a modified form of Desoxycholate Citrate Agar[1] which includes sucrose in its formulation. The addition of this fermentable carbohydrate increases the usefulness of the medium because non-pathogenic sucrose-fermenting organisms may be recognised by their red colonies, e.g some *Proteus,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

DCLS Agar reduces the number of false-positive sub-cultures 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.*

Typical growth of Salmonella (arrowed) on DCLS agar.



DCLS Agar may be inoculated directly from the specimen, or inoculated after enrichment through Selenite Broth CM395 and L121, Muller-Kauffmann Tetrathionate Broth CM343 or Tetrathionate Broth CM29. The plates should be incubated overnight (18-24 hours) at 35°C and examined for the presence of pale, translucent or colourless colonies. Sub-cultures can be made into confirmatory media such as Kligler Iron Agar CM33 or Triple Sugar Iron Agar CM277 or picked for transfer to nutrient broth for subsequent motility and agglutination 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 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

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.

References

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

Desoxycholate Citrate Agar

Code: CM35

A modification of Leifson's medium for the isolation of *Salmonella* and *Shigella* species.

Formula (grams per 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 48.5g 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.

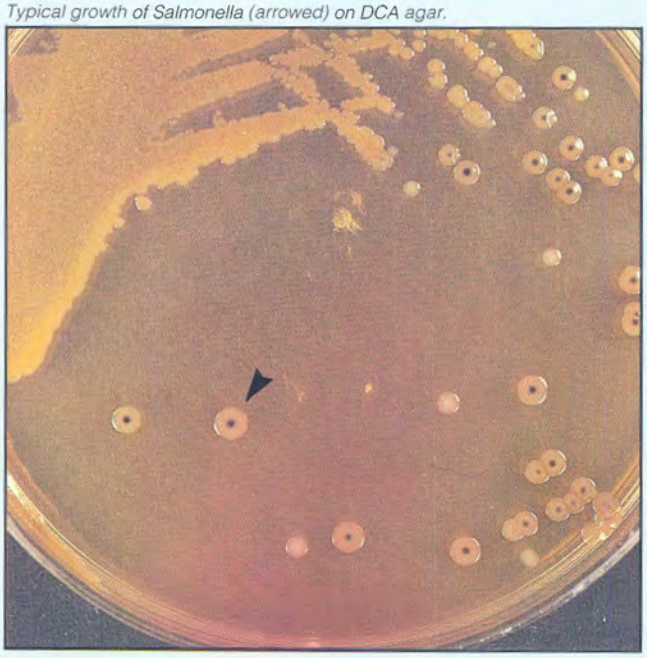
DO NOT AUTOCLAVE, OR REMELT.

Description

An Oxoid modification of Leifson medium, 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 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.

Typical growth of Salmonella (arrowed) on DCA agar.



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 Shigella are being sought as well as Salmonella[1].

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:
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 DCS 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 Fricker C.R. (1987) *J. Appl. Bact.*, **63,** 99-116.

Desoxycholate Citrate Agar (Hynes)

Code: CM227

A selective medium for the isolation of *Salmonella* and *Shigella* species.

Formula (grams per litre)

'Lab-Lemco' powder	*5.0*
Peptone	*5.0*
Lactose	*10.0*
Sodium citrate	*8.5*
Sodium thiosulphate	*5.4*
Ferric citrate	*1.0*
Sodium desoxycholate	*5.0*
Neutral red	*0.02*
Agar	*12.0*

pH 7.3 ± 0.2

Directions

Suspend 52g in 1 litre of distilled water. Bring to the boil over a 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.

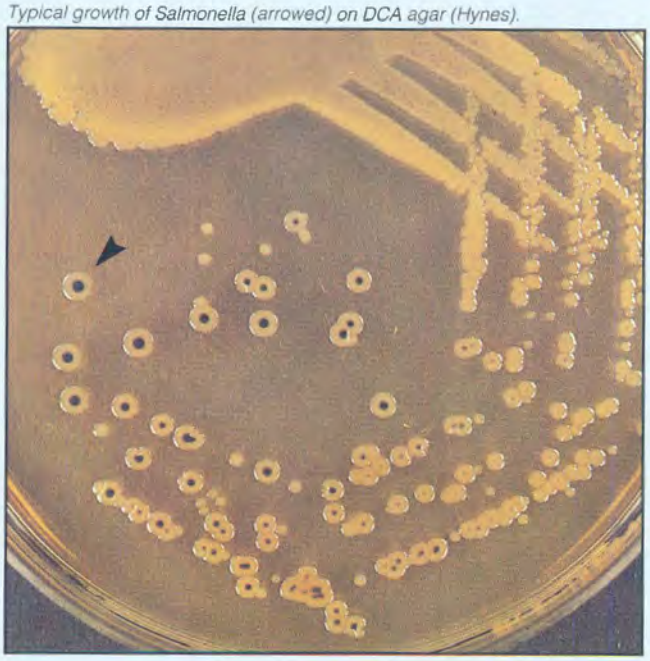
The improvement gives larger and more numerous colonies of *Shigella* species which can easily be picked off and emulsified in saline for slide agglutination tests.

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.

Typical growth of Salmonella (arrowed) on DCA agar (Hynes).



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 on to 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 1-2mm in diameter which may be surrounded by a zone of precipitation. *Aerogenes* colonies are domed and mucoid.

Shigella sonnei – the colonies grow from 1mm diameter after 18 hours incubation to 2mm 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 round a central dome.

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

Salmonella typhi – 0.25 to 1mm in diameter after 18 hours and pale pink, a day later they are flat, conical, 2mm 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 control:
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.

Hektoen Enteric Agar

Code: CM419

A differential, selective medium for the isolation of *Shigella* and *Salmonella* species.

Formula (grams per 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 76g 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 60°C and pour plates.

Description

Hektoen Enteric Agar was developed by King & 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 & 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 15mg/litre to improve the selectivity of the medium by inhibiting *Citrobacter* and *Proteus* species.

Hektoen Enteric Agar meets the requirements of the APHA[4].

Typical growth of Salmonella (arrowed) on Hektoen agar.

Technique

Inoculate the medium with fresh faeces suspended in Ringers solution or inoculate directly with rectal swabs. Food samples should be inoculated from the enrichment culture. 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 conolies.
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

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,** 577-561.
2 Taylor W.I. and Schelhaut D. (1971) *Appl. Microbiol*, **21,** 32-37.
3 Hoben D.A., Ashton D.H.A. and Peterson A.C. (1973) *Appl. Microbiol.*, **21,** 126-129.
4 American Public Health Association (1976) *Compendium of Methods for the Microbiological Examination of Foods. APHA Inc. Washington D.C.*

MLCB Agar

Code: CM783

Mannitol Lysine Crystal Violet Brilliant Green Agar for the selective isolation of Salmonellae (not *S. typhi* or *S. paratyphi A.*).

Formula (grams per litre)

Yeast extract	*5.0*
Peptone	*10.0*
'Lab-Lemco' powder	*2.0*
Sodium chloride	*4.0*
Mannitol	*3.0*
L-lysine hydrochloride	*5.0*
Sodium thiosulphate	*4.0*
Ferric ammonium citrate	*1.0*
Brilliant green	*0.0125*
Crystal violet	*0.01*
Agar	*15.0*
pH 6.8 ± 0.1	

Directions

Suspend 49.0 grams in 1 litre of distilled water. Mix and bring gently to the boil with frequent agitation to dissolve the medium completely. Cool to 50°C and pour approximately 20ml into sterile petri dishes.

DO NOT AUTOCLAVE OR OVERHEAT.

Description

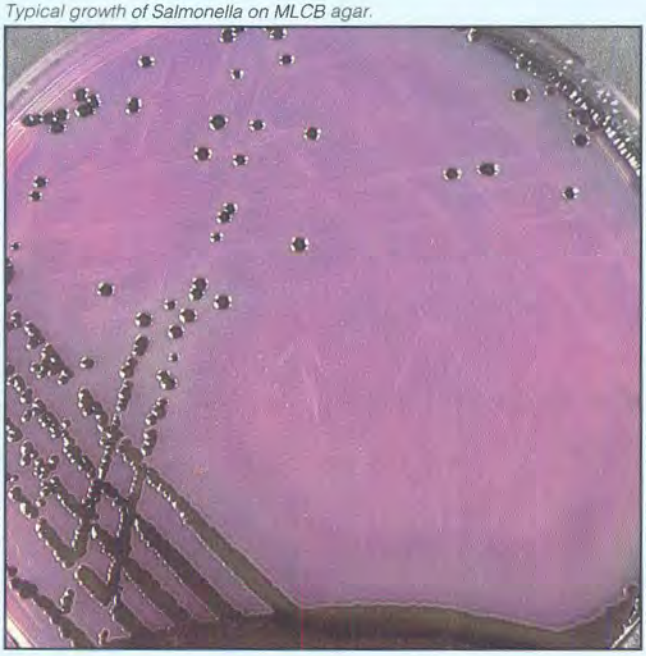
Mannitol Lysine Crystal Violet Brilliant Green Agar (MLCB Agar) CM783 is based on the formula of Inoue et al.[1] for the selective isolation of salmonellae from faeces and foods. Visual detection of very small numbers of hydrogen sulphide producing strains is easy because of the distinctive colonial appearance.

The concentration of Mg++ appears to be critical for maximum growth of salmonellae on MLCB Agar. van Schothorst et al.[2] showed that Oxoid MLCB Agar did not inhibit any of the *Salmonella* species investigated.

Salmonella serotypes that have a high incidence of H_2S negative strains e.g *S. sendai, S. berta, S. pullorum* and *S. seftenberg* may produce atypical pale colonies. MLCB Agar is not suitable for *S. typhi* and *S. paratyphi A.* because of the inhibitory concentration of brilliant green.

The medium may be inoculated directly with the specimen or from an enrichment culture. Selectivity is relatively weak and performance may be adversely effected by heavily contaminated specimens. Because of these limitations MLCB Agar should not be used alone.

Typical growth of Salmonella on MLCB agar.



van Schothorst et al.[2] reported MLCB Agar to be excellent for the isolation of H_2S+ve salmonellae after enrichment in Rappaport-Vassiliadis(RV) Enrichment Broth CM669. They found that the selectivity of MLCB Agar was substantially increased after RV broth enrichment. They suggested Brilliant Green Agar and MLCB Agar should be used when examining heavily contaminated samples.

Salmonellae grow as large purple-black colonies due to hydrogen sulphide production. Mannitol is utilised by the organism and the resultant pH fall initiates lysine decarboxylation which controls further downward pH movement and promotes blackening.

MLCB Agar does not depend on lactose fermentation and is therefore recommended when investigating lactose-fermenting salmonellae (*Salm. arizona*).

Atypical *Salmonella* strains that produce little or no hydrogen sulphide grow as mauve-grey colonies and may develop a central black 'bulls-eye'.

To assist the detection of these atypical strains Brilliant Green Agar (modified) CM329 or Bismuth Sulphite Agar CM201 should also be used.

Gram positive and most Gram negative organisms are inhibited although some strains of *Citrobacter* species may grow sufficiently well to mimic the appearance of *Salmonella* species and some *Proteus* species may swarm.

Most contaminating organisms that are able to grow develop as small colourless colonies.

Technique

Dry the surface of the agar before use.

Inoculate the medium heavily with the specimen or enrichment culture and incubate for 18-24 hours at 35°C.

Examine for typical large purple-black colonies of H_2S positive salmonella. Search carefully for H_2S negative strains that atypically grow as large mauve-grey colonies with a cratered centre. A proportion may show a black 'bulls-eye'.

Pick all colonies presumed to be *Salmonella* species and confirm by biochemical and serological testing.

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 of medium at 2-8°C.

Quality Control

Positive control:
Salmonella virchow NCTC® 5742

Negative control:
Escherichia coli ATCC® 25922

Precautions

The identity of colonies presumed from their appearance to be *Salmonella* species must be confirmed by biochemical and serological testing. In common with other enteric media care must be taken to ensure the purity of colonies taken for further testing as organisms that are inhibited from developing into colonies remain viable and may accidentally be picked on sub-culture.

References

1 Takao Inoue et al. (1968) Proceedings of the Japanese Society of Veterinary Science. Number 169. *Jap. J. Vet. Sci.*, **30.**

2 van Schothorst M., Renaud A. and van Beek C. (1987) *Food Microbiol*, **4,** 11-18.

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 per 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 63g 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.

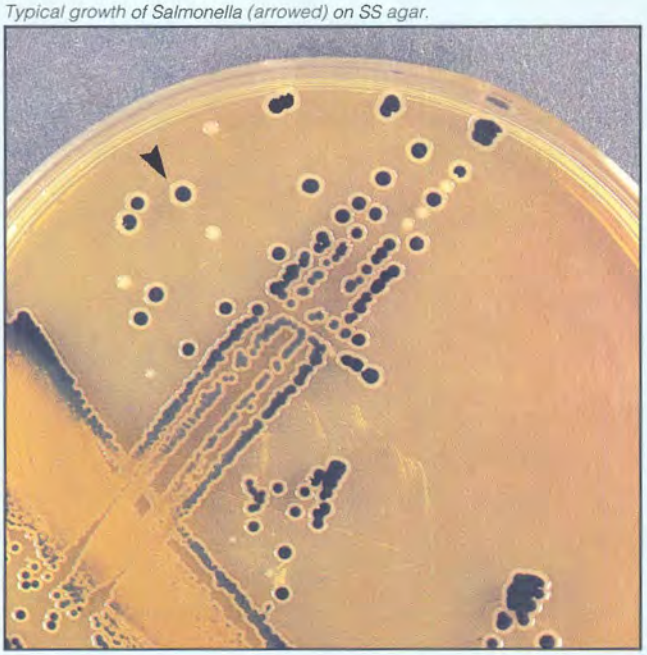
Cool to about 50°C, mix and pour into Petri dishes.

Description

SS Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella* species from pathological specimens, suspected foodstuffs, etc. Gram-positive and coliform organisms are inhibited by the action of the selective inhibitory components brilliant green, bile salts, thiosulphate and citrate.

Thiosulphate in combination with iron also acts as an indicator for sulphide production, which is indicated by blackening in the centres of the colonies.

Typical growth of Salmonella (arrowed) on SS agar.



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 18-24 hours at 35°C; non-lactose fermenters form colourless colonies, whilst occasional resistant coliforms or other lactose fermenters produce pink or red colonies.

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 to 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 NCTC® 13076
Shigella sonnei ATCC® 25931

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[1,2].

References

1 Liefson E. (1935) *J. Path. Bact.*, **40,** 581-
2 Taylor W.I. and Harris B. (1965) *Am. J. Clin. Path*, **44,** 476-

Salmonella Shigella Agar (SS Agar Modified)

Code: CM533

An improved formulation which gives better growth of Shigellae and better colony characteristics for Salmonellae.

Formula (grams per litre)

'Lab-Lemco' powder	*5.0*
Peptone	*5.0*
Lactose	*10.0*
Bile salts	*5.5*
Sodium citrate	*10.0*
Sodium thiosulphate	*8.5*
Ferric citrate	*1.0*
Brilliant green	*0.00033*
Neutral red	*0.025*
Agar	*12.0*
pH 7.3 ± 0.2	

Directions

Suspend 57g 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 and pour into Petri dishes.

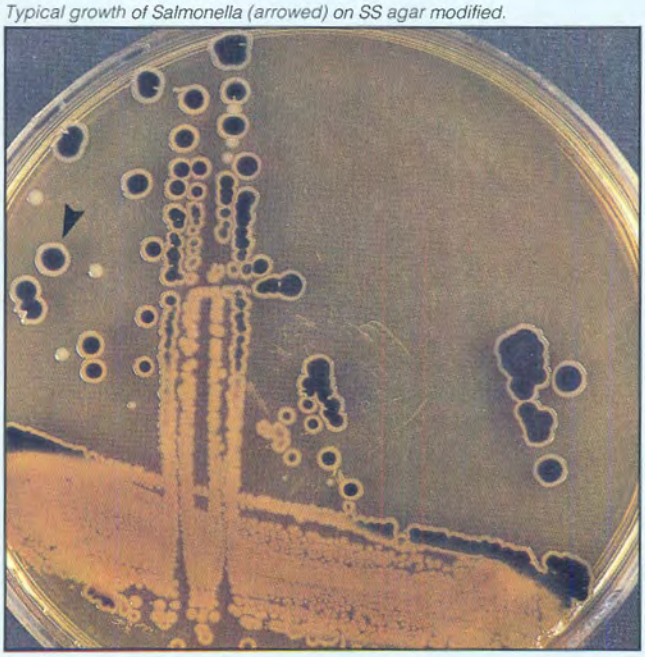
Description

Although widely used, SS Agar has been criticized because of excessive inhibition of *Shigella* species.

Investigation has shown that modification to the formulation by alterations to the bile salt mixture, peptone and pH value considerably improve its performance in the growth of shigellae without too much increased growth of commensal organisms.

Salmonella colonies are also larger with improved blackening at the centre.

Typical growth of Salmonella (arrowed) on SS agar modified.



The change in formulation has reduced the number of gm/litre from 63g to 57g.

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 18-24 hours at 35°C; non-lactose fermenters form colourless colonies. Occasional resistant coliforms and other lactose fermenters produce pink or red colonies.

In addition to the SS Agar (Modofied) plate, inoculate a tube of Selenite Broth enrichment medium, CM395. Incubate it for 12 hours at 35°C, and subculture on to another SS Agar (Modified) plate.

Colonial Characteristics

Non-Lactose Fermenting Organisms

Salmonella species
Transparent colonies usually with black centres
Shigella species
Transparent colonies
Proteus species
Citrobacter species
Transparent colonies with grey-black centres

Late-lactose fermenting organisms will develop colonies with pink centres after 48 hours incubation.

Storage conditions and Shelf life

As for SS Agar CM99.

Quality Control

As for SS Agar CM99.

XLD Medium

Code: CM469

A selective medium for the isolation of Salmonellae and Shigellae from clinical specimens and foods.

Formula (grams per litre)

Yeast extract	*3.0*
L-Lysine HCl	*5.0*
Xylose	*3.75*
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 53g 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

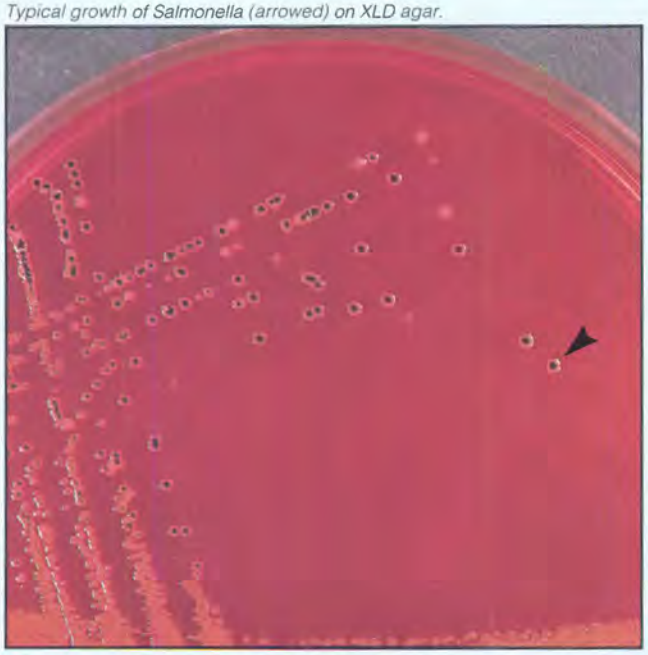
Xylose Lysine Desoxycholate Medium was originally formulated by Taylor[1] for the isolation and identification of shigellae from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both salmonellae and shigellae[2].

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[1]. Xylose is thus included in the medium so that *Shigella* species may be identified by a negative reaction.

Salmonella species 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 *Salmonella* and *Edwardsiella* species is differentiated from that of shigellae by a hydrogen sulphide indicator.

Typical growth of Salmonella (arrowed) on XLD agar.



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 coliform without decreasing the ability to support shigellae and salmonellae.

The recovery of *Shigella* species has previously been neglected despite the high incidence of shigellosis. This has been due to inadequate isolation media[3]. The sensitivity and selectivity of XLD Medium 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 Medium and these other media have been recorded in the literature[4,2,5,6,7,8,9,10].

The recovery of Salmonellae and Shigellae is not obscured by profuse growth of other species[3] therefore XLD Medium is ideal for the screening of samples containing mixed flora and suspected of harbouring enteric pathogens, e.g medical specimens or food products. Chadwick, Delisle & Byer[11] recommended the use of this medium as a diagnostic aid in the identification of Enterobacteriaceae.

Technique

Faeces or rectal swabs may be plated directly[12] 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°C for 18 to 24 hours.

Colonial Appearances

Organism	*Appearance*
Salmonella *Edwardsiella*	Red colonies with black centres
Shigella *Providencia* H_2S-negative Salmonella (e.g *S. paratyphi A)*	Red colonies
Escherichia *Enterobacter* *Klebsiella* *Citrobacter* *Proteus* *Serratia*	Yellow, opaque colonies

Note

False positive, red colonies may occur with some *Proteus* and *Pseudomonas* species.

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

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