WHO Global Foodborne Infections Network

(formerly WHO Global Salm-Surv)

"A WHO network building capacity to detect, control and prevent foodborne and other enteric infections from farm to table"

Laboratory Protocol "Isolation of *Salmonella* spp. From Food and Animal Faeces "

5th Ed. June 2010

IMPORTANT NOTES:

1) This procedure is based on the ISO protocol: 6579:2002 “Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp.”[4]. This protocol is intended to provide guidance for the testing of suspect food items/ animal faecal specimens identified via foodborne disease surveillance programmes. Regulatory agencies (Ministries of Health, Agriculture, Commerce, etc) have specific testing requirements, different from this protocol, which much be used to test samples collected for regulatory testing (example: import/export or product recall). Prior to performing any official, legal, or regulatory testing, the reader should confirm the appropriate protocol through consult with in-country regulatory authorities.

2) This protocol is intended only to be used on food samples and animal faeces. This protocol should not be used for the testing of human faeces.

Foreword:

Infections due to *Salmonella* spp. remain a global problem. These infections may cause significant morbidity and mortality both in humans and production animals as well as considerable economic losses. *Salmonella* spp. are typically transmitted among humans and animals via a fecal-oral route, usually through the consumption of contaminated food or water. Timely identification and serotyping of *Salmonella* from clinical specimens facilitates outbreak detection and patient management while prompt and accurate detection of *Salmonella spp.* in contaminated food or water provides an opportunity to prevent the contaminated food from entering the food supply.

Sensitive and specific laboratory methods for the isolation, identification, and serotyping of *Salmonella* are essential elements of *Salmonella* monitoring and control programmes. An ideal method will be rapid, inexpensive, easily reproducible, sensitive, and specific. Currently, no single method meets all these criteria and the optimal method may vary depending on the source of specimen (e.g., human clinical specimens, different food matrices, and environmental specimens) and the target serotype (e.g. typhoidal versus non-typhoidal *Salmonellae*). Additionally, new methods are being described regularly and comparison of current methodologies to new methodologies is highly recommended. To insure continuity of results, any new method must be validated and standardized prior to implementation.

The following protocol is based on the ISO-6579 standard method for the isolation and identification of *Salmonella* from food and animal faeces. This procedure has been extensively documented in the peer-reviewed literature and has been accepted by international accreditation agencies. It can be tailored to fit the needs of most laboratories world-wide. Molecular methodologies have the potential to increase sample throughput, sensitivity, and specificity, while simultaneously reducing turnaround time. However, it is important to utilize the appropriate test for the appropriate sample, for example PFGE can provide supplementary discrimination below the level of serotype, however it should not take the place of serotyping. Also, many molecular assays are non-culture tests (or only require pre-enrichment). While these assays may provide rapid screening results, it is essential that an attempt be made to obtain an isolate for additional studies such as serotyping and susceptibility testing.

Detailed identification and serotyping procedures may be found in the Global Salm-Surv Laboratory Protocol “Identification of *Salmonella* and *Shigella* Using an Abbreviated Panel of Tests”[7] and “Serotyping of *Salmonella*” and a flow diagram providing a brief overview of the isolation, identification, and serotyping procedure may be found in Appendix 3.

Contents

1. Isolation, identification and serotyping of *Salmonella* from faeces and food

Introduction:

The following procedures will guide you through the steps necessary to isolate *Salmonella* from animal faeces or food.

Description of Genus[2,7]:

The genus *Salmonella* is comprised of two species *S. enterica* and *S. bongori*. *S. enterica* is further divided into six subspecies which are designated by name or Roman numeral:

***Salmonella enterica* subspecies**	
I	*Salmonella enterica* subsp. *enterica*
II	*Salmonella enterica* subsp. *salamae*
IIIa	*Salmonella enterica* subsp. *arizonae*
IIIb	*Salmonella enterica* subsp. *diarizonae*
IV	*Salmonella enterica* subsp. *houtenae*
VI	*Salmonella enterica* subsp. *indica*

The majority of human *Salmonella* infections are caused by *S. enterica* subspecies I. Additionally, several invasive *Salmonella* serotypes; Typhi, Paratyphi A, Choleraesuis, Dublin, and Paratyphi C are all *S. enterica* subspecies I serotypes.

Salmonella serotype is determined by the immunoreactivity of three surface antigens “O” (LPS), “H” (flagellin protein), and “Vi” (capsule). Between the two species of *Salmonella*, over 2,500 unique serotypes have been described and new serotypes are described regularly.

As is typical of all other *Enterobacteriaceae*, the *Salmonellae* are Gram-negative, oxidase negative, facultative anaerobes. The *Salmonellae* are Vogues-Proskauer (VP) negative, methyl red positive, and reduce nitrate to nitrite without the production of gas. The *Salmonellae* are typically indol and urease negative, although rare indol or urease positive strains may be encountered. *Salmonellae* are typically motile by means of peritrichious flagella; however, nonmotile variants may be encountered and the host-adapted avian pathogens *Salmonella* serotypes Pullorum and Gallinarum are always nonmotile. There is considerable phenotypic variation between the two species of *Salmonella* and the six subspecies of *S. enterica* which may be utilized for differentiation. Additionally, several serotypes (notably *Salmonella* serotypes Typhi, Paratyphi A, Choleraesuis, and Paratyphi C) have biochemical profiles which are unique from other *Enterobacteriaceae* and can be utilized to make a serotype level identification even in the absence of serology.

Isolation of *Salmonella* from Animal Faeces and Food[3,4,5,6]:

The isolation of *Salmonella* from animal feces may be complicated by several factors. Animals may be subclincally infected (i.e. not showing clinical signs of disease) and shedding small numbers of *Salmonellae* in their faeces. Additionally, the population of *Salmonellae* in feces is typically much lower than that of other enteric flora. Similarly, *Salmonella* populations in food samples may be stressed due to heat, pH, or salt content, or unevenly distributed through the food matrix. Several steps are taken to insure optimal recovery of *Salmonellae* from these samples:

1) Use a large sample volume (25g). This helps to insure accurate representation of the entire matrix.

2) Use a pre-enrichment step, such as growth in buffered peptone water before direct plating for *Salmonella*. This allows stressed or injured *Salmonellae* to recover before exposure to selective enrichment media.
3) Use selective media to preferentially recover *Salmonella*.
    a. Use Mueller-Kauffmann's Tetrathionate broth (TTmk) and Rappaport-Vassiliadis Soya Peptone broth (RVS) for pre-enrichment. TTmk provides good control of *Escherichia* while RVS provides good control of *Proteus* and *Pseudomonads*.
    b. Use Xylose-Lysine Desoxycholate agar (XLD) and Brilliant Green agar (BGA) for direct plating.

The protocol presented here is based on the ISO-6579 standard and is intended for use with animal faeces and food products intended for consumption by humans or production animals. It should be noted that the media used in this protocol are highly selective and may be inhibitory to some typhoidal *Salmonellae* (particularly *Salmonella* serotypes Typhi and Paratyphi A). *Salmonella* serotypes Typhi and Paratyphi A are host adapted human pathogens, under normal circumstances these serotypes are not be found in animal faeces and are infrequently implicated in foodborne outbreaks. However, if testing of food samples for *Salmonella* Typhi or Paratyphi A is performed, it is essential to supplement this procedure with both a selective enrichment broth and selective plating media which do not inhibit these serotypes (e.g. selenite-cysteine broth and desoxycholate agar or bismuth sulphate agar)[3].

Safety[1]:

Several countries follow the CDC/NIH biosafety recommendations published in the "Biosafety in Microbiological and Biomedical Laboratories" 5th Ed (BMBL-5) (check year). The BMBL recommends BSL-2 practices and procedures when working with serotypes other than *Salmonella* Typhi.

BSL-2 procedures are recommended for the routine manipulation (e.g. processing clinical samples) of *Salmonella* Typhi and BSL-3 practices and procedures are recommended when working with production quantities of *Salmonella* Typhi or performing procedures likely to generate aerosols.

References

1) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Ed. 2007. Centers for Disease Control and Prevention, Atlanta, GA. Available at: http://www.cdc.gov/biosafety/publications/BMBL_5th_Edition.pdf

2) Brenner, F.W., & A.C. McWhorter-Murlin. 1998. Identification and Serotyping of *Salmonella*. Centers for Disease Control and Prevention, Atlanta, GA.

3) Health Protection Agency (2007). *Detection of Salmonella species.* National Standard Method F 13 Issue 3. http://www.hpa-standardmethods.org.uk/pdf_sops.asp.

4) ISO-6579 : 2002 (E) 4th Ed. Microbiology- General Guidance on Methods for the detection of *Salmonella*, International Organisation for Standardization, Geneve, Switzerland.

5) NMKL method no. 71, 2nd ed., 1999: Salmonella. Detection in food. Nordic committee on food analysis.

6) Post D. E. (1997) Food-borne pathogens monograph number I Salmonella. Oxoid limited, Hampshire, England.

7) WHO Global Foodborne Infections Network. Laboratory Protocol: "Biochemical Identification of *Salmonella/Shigella* Using an Abbreviated Panel of Tests" January 2010. Available at: http://www.antimicrobialresistance.dk/data/images/protocols/gfn_biochem_final.pdf

1.1 Isolation of *Salmonella* from food and animal faeces.

Materials

Equipment

- Erlenmeyer flasks (500 ml) etc. sterile (for pre-enrichment)
- Disposable inoculation loops (1 μl and 10 μl)
- Plastic petri dishes (9 cm diameter) sterile
- Balance
- Incubators at 37^{o}C and 41.5^{o}C
- Bunsen burner
- Pipettes for 0.1 ml (e.g. 1 ml pipettes)
- Wood spatulas

Media

- Buffered peptone water 225 ml
- Tetrathionate broth (Müller-Kauffmann) 10 ml
- Rappaport Vassiliadis soy peptone broth 10 ml
- Xylose Lysine Desoxycholate (XLD) agar plates
- Brilliant Green (BGA) agar plates
- Nutrient agar plates

Bacterial strains

- Food samples
- Animal Faecal samples

Safety

Carry out all procedures in accordance with the local codes of safe practice.

Specimen Collection and Transport:

Samples may consist of food or animal faeces.

Ideally, at least 25g of food or animal faeces should be submitted. However, smaller samples may be submitted if larger samples cannot be obtained.

Salmonella spp. may not be evenly distributed within a sample. Specimens should be mixed prior to testing and specimens should be obtained from several locations within the sample.

Food samples should be transported to the laboratory at the appropriate temperature. Foods should be maintained at their recommended storage temperature during transport: Frozen foods (example: ice cream) should be remain frozen for transport; cold foods (example: milk) should be kept cold (not frozen) for transport; and room temperature foods (example: powdered formula) should be transported at room temperature.

Faecal samples must be submitted in a clean, container with no soap or disinfectant residue. Small faecal samples (example: swabs from small animals) may be placed in transport media. The sample must be kept cold and transported to the lab within 8 hours of collection. If the sample cannot reach the laboratory within 8 hours; the sample should be frozen at < 70°C or stored on dry ice.

Procedure:

Day 1: Non-selective pre-enrichment
Weigh out 25 g food or animal faeces with a sterile wood spatula, place the sample into an Erlenmeyer flask and add 225 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Mix. Incubate at 36°C (+/- 1°C) overnight (16-20 hours).

Day 2: Prepare selective enrichment (I) and (II)
Use a pipette to transfer 1 ml of the pre-enrichment broth to 10 ml Tetrathionate broth (Müller-Kaufmann). (Label as Tube I)

Use a micro-pipette to transfer 0.1 ml (100 uL) of the pre-enrichment broth to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth. (Label as Tube II)

Incubate Tube I: Tetrathionate broth (Müller-Kaufmann) at 36.0°C ± 1°C and Tube II: Rappaport-Vassiliadis soy peptone (RVS) at 41.5°C± 0.5°C overnight (18-24 hours).

Day 3: Spread on selective agar plates
Spread a 10 µl loop full from the inoculated and incubated Tetrathionate broth (I) and RVS broth (II) on XLD and on BGA agar plates and incubate at 36.0°C ± 1°C overnight (18-24 hours).

Theory / Comments:

The volume of the food or faeces sampled determines the sensitivity of detection. Sensitivity increases with larger sample volumes. In practice, however, laboratories may receive samples smaller or larger than 25g. While not ideal, it is not uncommon for swabs (volume less than 1 gram) to be submitted. Or to receive large samples in excess of 25 grams.

It is critical that the ratio of 1 part sample plus 9 part buffered peptone water be maintained. When small samples are received, the volume of buffered peptone water must be decreased. To maintain the ratio of 1 part sample plus 9 parts buffered peptone water.

With large samples, the sample should be completely mixed and a 25g sample should be obtained by collecting small portions from various areas of the sample. Alternatively, the sample can be divided into 25g segments and inoculated into 2 or more bottles of buffered peptone water.

This ratio also applies to pooled samples, for instance 5x5 grams of faeces to 225 ml pre-enrichment broth.

Procedure:

Day 4: Selection and Subculture of Suspect *Salmonella* Colonies

Examine the XLD plates:

A typical *Salmonella* colony has a slightly transparent red halo and a black centre, a pink-red zone may be seen in the media surrounding the colonies. Note the presence of typical *Salmonella*- like colonies on XLD with a + in the record sheets.

Examine the BGA plates:

Typical *Salmonella* colonies on a BGA agar plate appear red and impart a red/pink colour to the surrounding agar. Other enterics typically appear green or yellow. Note the presence of typical *Salmonella*-like colines on BGA with a + in the record sheets.

Plate two suspect colonies from XLD agar and BGA onto non-selective media (e.g. nutrient agar) for biochemical confirmation and serotyping.

Day 5-7: Biochemical Identification and Serotyping:

Please refer to WHO GFN Procedures "Identification of *Salmonella* and *Shigella* using an Abbreviated Panel of Tests" and "Serotyping of *Salmonella*".

4. Composition and preparation of culture media and reagents

If no reference is given, it is the procedure used at DVL.
The media and reagents are available from several companies including Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturers description if it differs from the description given here. Refer to Appendix 2 for a colour presentation of growth of *Salmonella* on selective agar media and positive and negative reactions of biochemical tests.

Brilliant Green Agar (BGA) (ref. 1)

Formula of CM263 from Oxoid

Proteose peptone	10.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Phenolred	0.09 g
Brilliant green	0.0047 g
Agar	12.0 g
Water	1000 ml

Preparation:
Dissolve 50g of the dehydrated medium in water by heating to the boiling point for 1 minute, adjust pH to 6.7 - 7.1 if necessary and transfer to sterile 1000 ml bottles. Do not autoclave.

Description:
Brilliant green is a selective agent. Its indicative principle is based on the ability to ferment lactose and sucrose. Phenol red is the pH indicator, which changes from yellow to red at pH 6.8 - 8.4. Therefore, lactose negative and sucrose negative bacteria like *Salmonella* grow as red-pink, white opaque colonies surrounded by brilliant red zones in the agar.
Proteus and *Pseudomonas* species may grow as small red colonies. Lactose and/or sucrose fermenting organisms are normally inhibited but may grow as yellow to greenish-yellow colonies surrounded by intense yellow-green zones in the agar. These may belong to *E. coli* or the *Klebsiella/Enterobacter* group.

Buffered peptone water (ref. 2)

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.5 g
Water	1000 ml

Preparation:
Dissolve the peptone and chemicals in water, adjust pH to 7.0 after sterilisation. Dispense into suitable flasks and autoclave at 121^{o}C for 20 min.

Nutrient agar (ref. 2)

Meat extract	3.0 g
Peptone	5.0 g
Agar	12 g to 18 g[1)]
Water	1000 ml

[1)] Depending on the gel strength of the agar.

Preparation:
Dissolve the dehydrated medium in the water by heating if necessary. Adjust pH to ~7.0 after sterilisation, transfer into bottles and autoclave at 121°C for 20 min. Pour 15 ml of melted medium in each plate.

Rappaport-Vassiliadis Soy Peptone (RVS) Broth (ref. 3)

Base

Soy peptone	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.4 g
Dipotassium hydrogen phosphate (K_2HPO_4)	0.2 g
Distilled water	1000 ml

Heat to about 80°C to dissolve all ingredients. Prepare this solution the same day as the complete RVS medium is prepared.

Magnesium chloride solution

Magnesium chloride ($MgCl_2 \cdot 6H_2O$)	400 g
Water	1000 ml

Dissolve the salt in the water. Because this salt is very hygroscopic, it is advisable to dissolve the entire contents of a newly opened container in distilled water. The magnesium chloride solution can be stored unsterilised, in a dark bottle with screw cap, at room temperature for up to 2 years.

Malachite green solution

Malachite green oxalate	0.4 g
Distilled water	100 ml

Dissolve the salt in the water. The solution can be stored unsterilised, in a dark bottle with screw cap, at room temperature for up to 8 months.

Complete medium

Base	1000 ml
Magnesium chloride solution	100 ml
Malachite green solution	10 ml

Preparation:
Mix the solutions well and distribute the solution in portions of 10 ml per tube with screw cap. Autoclave at 115°C for 15 min. Adjust the pH so that after sterilisation it is 5.2 ± 0.2 at 25°C. Store at about 4°C for a maximum of 4 months.

Description:
This medium is used as a selective enrichment medium for the isolation of *Salmonella* from food, environment specimens and from faeces. Malachite green is the selective agent.
Salmonella species have the following characteristics when compared with other *Enterobacteriacea.*

- Ability to survive at relatively high osmotic pressure
- Multiply at relatively low pH values
- Are more resistant to malachite green and have less demanding nutritional requirements.

The medium is not useful while *Salmonella* Typhi is suspected.

Tetrathionate broth

Brilliant green solution

Brilliant green	0.1 g
Sterile distilled water	100 ml

Iodine - Potassium iodine solution

Iodine double sublimated	16 g
Potasium iodide z.A.	20 g
Sterile distilled water	80 ml

Base (e.g. Tetrathionate Anreicherings bouillon nach Mueller Kauffman from Merck, no. 10863)

Meat extract	0.9 g
Peptone from meat	4.5 g
Yeast extract	1.8 g
Sodium chloride	4.5 g
Calcium carbonate	25.0 g
Sodium thiosulfate	40.7 g
Ox bile, dried	4.75 g
Sterile water	1000 ml
Brilliant green solution 1:1000	10 ml
Iodine-Potassium iodine solution	20 ml

Preparation:
Dissolve the tetrathionate bouillon in sterile water in a flask by shaking. Aseptically add brilliant green solution and then iodine-potassium iodine solution. Adjust pH to 7.4 - 7.8 at 25°C. Store bouillon at about 4°C.

Description:
Tetrathionate broth is used for selective enrichment of *Salmonella*. According to ref. 2 Mueller-Kauffman Tetrathionate broth (CM343) has improved selectivity compared with Tetrathionate broth (USP) (CM671) and Tetrathionate broth (CM29) all from Oxoid, but it is inhibitory to *S*. Typhi, *S*. Pullorum, and *S*. Gallinarum (ref. 1).

Xylose lysine desoxycholate (XLD) agar (ref. 3)

Yeast extract	3.0 g
Sodium chloride	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
L-lysine hydrogen chloride	5.0 g
Sodium thiosulphate	6.8 g
Iron(III)ammonium citrate	0.8 g
Phenol red	0.08 g
Sodium desoxycholate	1.0 g
Agar	15.0 g
Distilled water	1000 ml

Preparation:
Dissolve the components in the water. Heat under constant stirring until the medium starts to boil. Avoid over-heating. Avoid preparing too large a volume of medium, as this requires prolonged heating. Immediately transfer the solution to a water bath at about 50°C, continue stirring until the medium has reached about 50°C. Adjust the pH so that after heating it is 7.4 ± 0.2 at 25°C. Poured agar plates can be stored for a maximum of 14 days, if stored in plastic bags in the dark at about 4°C.
Add 10 ml of a 0.15% filter sterilised solution of sodium novobiocin to increase the selectivity.

Description:
Sodium desoxycholate is the selective agent and phenol red is the pH indicator. The indicative principle is based on lactose, sucrose and xylose fermentation, H_2S production and lysine decarboxylation. If H_2S is produced from sodium thiosulphate, black FeS (Ferrosulfide) will develop. *Salmonella* ferments xylose, but not lactose and sucrose, decarboxylate lysine and produces H_2S. *Salmonella* suspect colonies grow as red colonies with a black centre. Other bacteria that may grow on XLD agar are usually yellow and the agar will also turn yellow. Other bacteria such as *Edwardsiella* may mimic *Salmonella*.

References

1. Post D. E. (1997) Food-borne pathogens monograph number I *Salmonella*. Oxoid limited, Hampshire, England.
2. ISO 6579 :1993(E) 3rd ed. Microbiology - General guidance on methods for the detection of *Salmonella.*
3. NMKL method no. 71, 2nd ed., 1999: *Salmonella*. Detection in food. Nordic committee on food analysis.

APPENDIX 1

Flow diagram for isolation/identification of *Salmonella* from Food / Animal Faceces

Non-selective pre-enrichment
25 g food / faeces in 10% phosphate buffer (36° +/-1°C, 24 h.)

↓

Selective enrichment *
0.1 ml in 10 ml Rappaport-Vassiliadis Soy Broth (41.5° +/- 0.5°C, 24 h.)
1 ml in 10 ml Tetrathionate broth (Müller-Kauffman) (36° +/-1°C, 24 h.)

↓

Isolation
XLD with an inoculation loop (36° +/-1°C, 24 h.)
BGA with an inoculation loop (36° +/-1°C, 24 h.)

↓

Suspect colonies to nutrient agar (36° +/-1°C, 24 h.)

↓

Biochemical confirmation (36° +/-1°C, 24 h.)
Triple Sugar Iron Agar (TSI) or Kliger Iron Agar (KIA)
Urea broth
Lysine Iron Agar (LIA)
Motility-Indol-Ornithine (MIO)
Citrate

↓

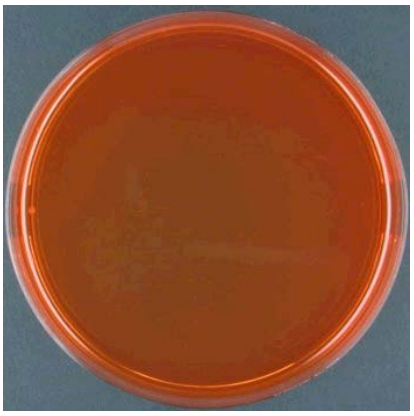
Serotyping
O-antigens
H-antigens
Phase I (37°C, overnight)
Phase II

* If *Salmonella* serovars Typhi or Paratyphi A are suspected: inoculate 1mL of pre-enrichment broth into 10mL of Selenite Cystine (or Selenite F) broth and incubate at 36° C (+/-1°C) for 18-24 h. Following incubation, it is advisable to inoculate the selective broth onto bismuth sulphate agar (in addition to XLD and BGA).

APPENDIX 2

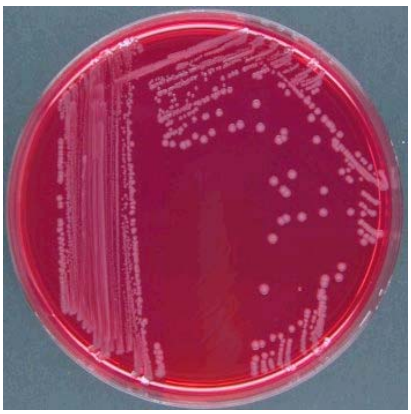
Photographs of *Salmonella* growth on various media and positive and negative reactions of biochemical tests.

Brilliant green agar (BGA) (e.g. from Oxoid).

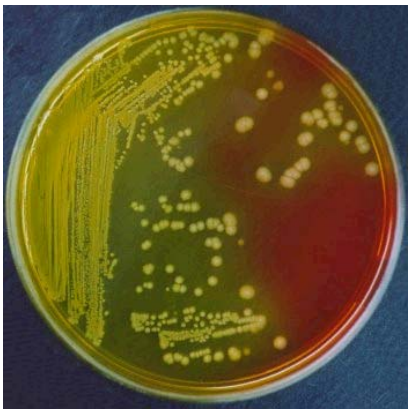


This medium is used to isolate *Salmonella* from pathological material, foodstuffs, etc. by separating lactose- and/or sucrose-positive bacteria from lactose- and sucrose-negative bacteria. Brilliant-green in the medium inhibits accompanying micro-organisms.

The picture shows an uninoculated plate.



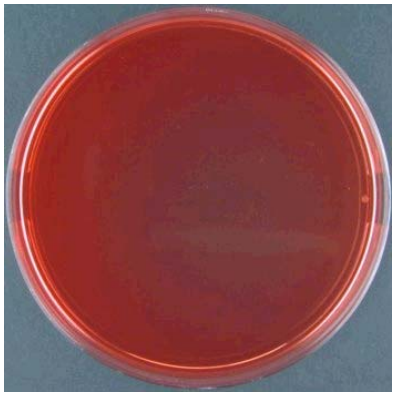
Salmonella on BGA Agar. The colonies are red because the bacterium does not ferment lactose or sucrose.

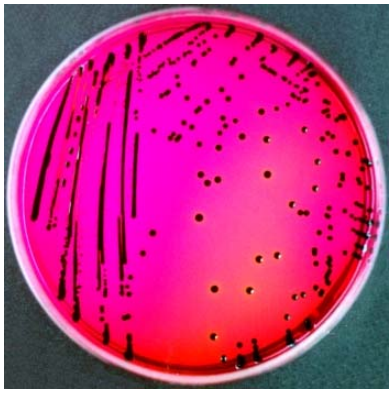


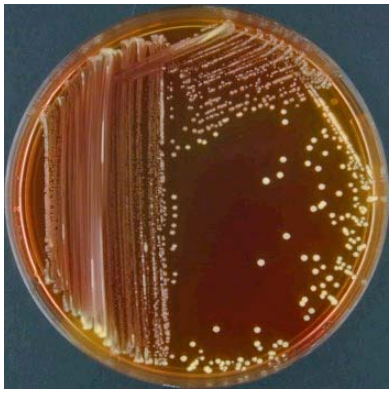
Escherichia coli on BGA Agar. The colonies are yellow due to the low pH which is caused by the production of acid during fermentation of lactose and/or sucrose.

Xylose lysine desoxycholate (XLD) agar

This medium is used for the isolation of shigellae and salmonellae from foods and clinical specimens. *Shigella*, *Providencia* and *Edwardsiella* do not ferment xylose, sucrose or lactose, therefore they alter the pH to alkaline and produce red colonies. *Salmonella* spp. ferments xylose, but at the same time decarboxylates lysine in the medium causing an alkaline pH and thus producing red colonies. The hydrogen sulphide producers, *Salmonella* and *Edwardsiella*, grow colonies with a black centre because the medium contains an iron salt. Fermentation of sucrose and/or lactose produces higher acid levels, thus preventing sucrose and/or lactose positive bacteria from reverting the pH to alkaline through decarboxylation of lysine. Non-pathogenic hydrogen sulphide-producing bacteria do not decarboxylate lysine. At the same time, the level of acid produced by fermentation prevents blackening of the colonies until after 18 or 24 hours. Sodium desoxycholate is an inhibitor which, at the concentration used in this medium, will inhibit coliforms but not salmonellae and shigellae.







Left to Right:
Uninoculated XLD plate; *Salmonella* spp. (clear colonies with black centres; note: serovars Typhi and Paratyphi A may produce clear colonies); *E. coli* (yellow colonies).

Date: _____ **Record sheet:**

Initials:_____ **Isolation of *Salmonella* from faeces and food.**

Morphology on selective agar plates

Sample: Faeces #1

	Colour	**Results**	**Comments**
From Tetrathionate: Morphology on BGA			
Morphology on XLD			
From RVS: Morphology on BGA			
Morphology on XLD			

Sample: Faeces #2

	Colour	**Results**	**Comments**
From Tetrathionate: Morphology on BGA			
Morphology on XLD			
From RVS: Morphology on BGA			
Morphology on XLD			

Date: _____ **Record sheet:**

Initials:_____ **Isolation of *Salmonella* from faeces and food.**

Morphology on selective agar plates

Sample: Food #1

	Colour	**Results**	**Comments**
From Tetrathionate: Morphology on BGA			
Morphology on XLD			
From RVS: Morphology on BGA			
Morphology on XLD			

Sample: Food #2

	Colour	**Results**	**Comments**
From Tetrathionate: Morphology on BGA			
Morphology on XLD			
From RVS: Morphology on BGA			
Morphology on XLD			