Global Salm-Surv

A global *Salmonella* surveillance and laboratory support project of the World Health Organization

Laboratory Protocols

Level 1 Training Course

Isolation of *Salmonella*

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Foreword

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.

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.

Surveillance of *Salmonella* in all the different stages of feed-food chain constitutes an important element in the exploration of epidemiology of foodborne salmonellosis, and in the development and implementation of efficient *Salmonella* control strategies.

Efficient laboratory methods for isolation, identification and typing of *Salmonella* are essential elements in *Salmonella* monitoring and control programmes. This protocol describes methods that have been extensively documented in the scientific literature, are accepted by international standardisation bodies and can be applied under most conditions and circumstances in laboratories worldwide.

It is, however, important to note the following: There are many different procedures for isolation of *Salmonella*. The ideal method has a high sensitivity and specificity, and at the same time is simple, rapid and inexpensive. No single method fulfils all these criteria, and no single method is optimal for all purposes. Therefore, it is advisable to consult the literature before choosing a method for a particular purpose. Frequently comparing your existing methods to newer methods is also highly advisable.

In this manual we present the methods for isolation of *Salmonella* according to the ISO-6579 standard but other methods may be used. The criteria for biochemical identification of *Salmonella* are relatively standard, however the format varies. Molecular methods are increasingly being introduced as an alternative. These methods often lead to a faster diagnosis and may be simpler to conduct, but they have the disadvantage that they may be expensive.

The *Salmonella* serotyping system is probably the best phenotypic bacterial typing system ever developed. It has a high discriminatory power and provides information that has great epidemiological significance. Molecular typing methods such as PFGE can yield supplementary information, but are so far not a substitute for serotyping or micro tray agglutination, etc. Availability and cost of high quality antisera can be a problem in some countries and regions.

In a survey of national *Salmonella* reference laboratories, conducted before the launch of Global Salm-Surv, laboratories were asked about which guidelines/standards they used for antimicrobial susceptibility testing. Nearly 3 out of 4 laboratories used the NCCLS guidelines. In this light, GSS has decided to promote the use of the NCCLS guidelines, but GSS laboratories are not obligated to follow these guidelines.
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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* confidently from faeces and from food, carry out a biochemical identification of *Salmonella* and serotype the strains using slide agglutination tests:

1. Isolation of *Salmonella* from food and faeces.
2. Biochemical confirmation of *Salmonella* suspect colonies (after isolation from faeces or food).
3. Serotyping of *Salmonella*.

The genus consists of two species: (1) *S. enterica* which is divided into six subspecies: *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *bongori*; and (2) *S. bongori* (formerly called *S. enterica* subsp. *bongori*). The subspecies name does not need to be indicated, as only serovars of subspecies *enterica* has a name. Therefore we write e.g. *Salmonella* Typhimurium.

*Salmonella* is generally identified as being a non-lactose fermenting, (NLFs) Gram negative rod shaped organism, ranging 0.7 to 1.5 x 2 to 5 µm in size. With the exception of *S. Pullorum* and *S. Gallinarum*, they are motile with peritrichous flagellae. D-glucose is fermented with the production of acid and usually gas. Other carbohydrates usually fermented are L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol (except ssp VI), trehalose, D-xylose and dulcitol.

*Salmonella* is oxidase negative, catalase positive, indole and Voges Proskauer (VP) negative, methyl red and Simmons citrate positive, H₂S producing and urea negative. Some of these characteristics are used for biochemical confirmation of *Salmonella*.

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

Some farm animals are infected with *Salmonella* without showing signs of the illness, i.e. they are subclinically infected. Faeces from these herds may contain *Salmonella* in low numbers. In food, *Salmonella* may also be present in low numbers in addition to a lot of other micro-organisms, and they may be injured. To diminish the risk of obtaining false negative results, a non-selective pre-enrichment of a large faeces or food sample, a combination of two selective enrichments and plating on two selective media are performed:

- Pre-enrichment in non-selective medium (buffered peptone water).
- Selective enrichment in Tetrathionate broth (Müller-Kauffmann) and Rappaport Vassiliadis soy peptone (RVS) broth.
- Subcultivation on Xylose Lysine Desoxycholate (XLD) agar and on Brilliant Green agar (BGA) (or another selective agar medium).

The ISO-6579 standard is applicable to products intended for human consumption or feeding of animals. The procedures for isolation of *Salmonella* from food and animal faeces given in this protocol follow the ISO-6579: 2002 standard (ref. 1). An overview of the procedure of isolating *Salmonella* according to other standards is given in Appendix 1.
It is advisable to include an extra selective pre-enrichment medium other than RVS as this is a very selective media and may not propagate some Salmonella spp. like Salmonella Typhi and Salmonella Paratyphi. In addition, RVS broth and XLD plates are already included in the Nordic standard for detection of Salmonella in food (ref. 2). Instead of BGA plates you may use other selective agar plates, e.g. Bismuth sulphite agar, Hektoen agar, Mannitol Lysine Crystal Violet Brilliant Green agar, Desoxycholate-citrate agar or Salmonella Shigella agar (ref. 3).

The manual does not contain a method for the isolation of Salmonella from human faeces, as it is not included in the training course.

**Biochemical confirmation and serotyping of Salmonella**

Subsequently it is confirmed with biochemical tests whether the colonies resembling Salmonella on XLD and BGA are Salmonella. The ISO-6579 standard (ref. 1) recommends using the TSI agar, Urea agar (Christensen), L-lysine decarboxylase, β-galactosidase (ONPG), Voges Proskauer and Indole tests in this order. These tests are described in this manual except that the urease test is performed with urea broth instead of urea agar. (According to ref. 2 Triple Sugar Iron (TSI) agar, mannitol, urea, ornithine decarboxylase and lysine decarboxylase could be suitable to confirm Salmonella suspect colonies).

In addition, the Salmonella colonies are serotyped and classified on subspecies level. The biochemical confirmation of Salmonella and the serotyping may be performed at the same time.

**Safety**

Several countries follow the CDC/NIH biosafety recommendations indicated in "Biosafety in Microbiological and Biomedical Laboratories", 4th Edition, 1999 (ref. 4) that recommend Biosafety Level 2 practices for all the Salmonella, except S. Typhi. For S. Typhi they recommend Biosafety Level 2 practices for activities with clinical materials and cultures; and Biosafety Level 3 practices for activities that can generate aerosols or for activities involving big volumes of organisms.

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

**References**

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°C and 41.5°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
- Faeces samples

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

**Day 1: Non-selective pre-enrichment**
Weigh out 25 g food or faeces with a sterile wood spatula, put it into an Erlenmeyer flask etc. and add 225 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Mix. Incubate at 37°C overnight (16-20 hours).

**Day 2: Prepare selective enrichment (I) and (II)**
I. Transfer 1 ml of the pre-enrichment with a pipette to 10 ml Tetrathionate broth (Müller-Kaufmann).
II. Transfer 0.1 ml of the pre-enrichment with a pipette to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth.

Incubate tube I at 37.0°C ± 0.5°C and tube II 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 37°C overnight (18-24 hours).

**Day 4: Subcultivation of Salmonella suspect colonies**
Read the XLD plates:
A typical Salmonella colony has a slightly transparent zone of reddish colour and a black centre, a pink-red zone may be seen in the media surrounding the colonies. Mark typical Salmonella growth on XLD with a + in the record sheets.

Read the BGA plates:
Typical Salmonella colonies on a BGA agar plate cause the colour of the medium to be red/pink (phenolred is the indicator). The colonies are grey-reddish/pink and slightly convex.
Mark typical Salmonella growth on BGA with a + in the record sheets.

**Theory / comments**

The volume of the faeces investigated determines the sensitivity of detection, the larger the volume the higher sensitivity. Volumes ranging from a swab sample of less than 1 gram to 25 grams are commonly applied. It is critical that the relationship between faeces and pre-enrichment broth remains 1 part faeces to 9 part buffer for all volumes. The more faeces the higher is the sensitivity (ref. 2). This ratio also applies to pooled samples, for instance 5x5 grams of faeces to 225 ml pre-enrichment broth.
Procedure

Plate 2 *Salmonella* suspect colonies on XLD and BGA agar onto non-selective media, e.g. nutrient agar plates for biochemical confirmation of *Salmonella* and serotyping.

**Day 5-7: Biochemical confirmation and serotyping of *Salmonella***

See the following procedures in the other manuals.
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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Proteose peptone</td>
<td>10.0 g</td>
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<tr>
<td>Yeast extract</td>
<td>3.0 g</td>
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<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Phenolred</td>
<td>0.09 g</td>
</tr>
<tr>
<td>Brilliant green</td>
<td>0.0047 g</td>
</tr>
<tr>
<td>Agar</td>
<td>12.0 g</td>
</tr>
<tr>
<td>Water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**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)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Peptone</td>
<td>10.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate dodecahydrate</td>
<td>9.0 g</td>
</tr>
<tr>
<td>(Na$_2$HPO$_4$.12H$_2$O)</td>
<td></td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate (KH$_2$PO$_4$)</td>
<td>1.5 g</td>
</tr>
</tbody>
</table>
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°C for 20 min.

**Nutrient agar (ref. 2)**

- Meat extract 3.0 g
- Peptone 5.0 g
- Agar 12 g to 18 g
- 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$_2$PO$_4$) 1.4 g
- Dipotassium hydrogen phosphate (K$_2$HPO$_4$) 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$_2$·6H$_2$O) 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**

<table>
<thead>
<tr>
<th>Base</th>
<th>1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium chloride solution</td>
<td>100 ml</td>
</tr>
<tr>
<td>Malachite green solution</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

**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 |
| Potassium 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)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Xylose</td>
<td>3.75 g</td>
</tr>
<tr>
<td>Lactose</td>
<td>7.5 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7.5 g</td>
</tr>
<tr>
<td>L-lysine hydrogen chloride</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Sodium thiosulphate</td>
<td>6.8 g</td>
</tr>
<tr>
<td>Iron(III)ammonium citrate</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.08 g</td>
</tr>
<tr>
<td>Sodium desoxycholate</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

**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, H₂S production and lysine decarboxylation. If H₂S is produced from sodium thiosulphate, black FeS (Ferrosulfide) will develop. *Salmonella* ferments xylose, but not lactose and sucrose, decarboxylate lysine and produces H₂S. *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**

APPENDIX 1
An overview of different standards for *Salmonella* isolation procedures

Figure 1. BSI/ISO *Salmonella* Isolation Procedure

**PRE-ENRICHMENT**
Test portion, 25g + buffered Peptone water, 225ml*

16-20 h, 37°C

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**SELECTIVE ENRICHMENT**
Culture, 0-1ml + Rappaport (RV) broth 10ml
18-24 h, 42°C (2 periods)

Culture, 10ml + Tetrathionate Broth (Müller Kauffman) 10ml
18-24 h, 42°C (2 periods)

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**SELECTIVE DIAGNOSTIC ISOLATION**
Plate on Brilliant Green Agar (Edel and Kampelmacher)
and any other solid selective medium†

24 h, 35°C or 37°C (48 h, if necessary)

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Pick five presumptive *Salmonella* colonies from each agar plate
and inoculate on nutrient agar

18-24 h, 35°C or 37°C

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**BIOCHEMICAL CONFIRMATION**

24 h, 37°C

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**SEROLOGICAL CONFIRMATION**
Slide agglutinations - O, Vi, H antisera

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* If the sample weight is less than 25 grams use the necessary quantity of medium to give a 1/10 dilution (weight to volume).
† The choice of a second medium is discretionary unless a specific medium is named in an International Standard relating to the product to be examined. It is advisable to include bismuth sulphite agar if *S. Typhi* or lactose fermenting salmonellae are suspected.
Figure 2
FDA/AOAC BAM Salmonella Isolation Procedure
Conventional Cultural Enrichment (FDA/BAM/AOAC)

PRE-ENRICHMENT
Test portion, 25g + pre-enrichment medium, 225ml*

24 ± 2 h, 35°C

SELECTIVE ENRICHMENT
Culture, 1ml +
tetrathionate broth 10ml

Culture, 1ml +
selenite cystine broth**, 10 ml or
Rappaport (RV) broth, 10 ml†

24 ± 2 h, 35°C

SELECTIVE DIAGNOSTIC ISOLATION
Plate on bismuth sulphite Agar. Xylose lysine desoxycholate agar.
Hektoen enteric agar

24 ± 2 h, 35°C
(48 h, if necessary)

BIOCHEMICAL CONFIRMATION
Pick two or more suspect colonies from each agar plate for biochemical tests

24-48 h, 35°C

SEROLOGICAL CONFIRMATION
Slide agglutinations - O,H antigens

Incubated pre-enrichment cultures and enrichment cultures of low-moisture foods may be refrigerated for up to 72 hours before plating on agar media¹.

* Lactose broth is commonly, but not always, used for pre-enrichment. The FDA Bacteriological Analytical Manual should be consulted for detailed procedures used with various types of food.

** Selenite is suspected to be carcinogenic

† Rappaport (RV) broth replaces Selenite cystine broth when examining shrimp, raw flesh foods, highly contaminated foods and animal foods.

Reference:
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.

The picture above shows an uninoculated plate.

*Salmonella* on XLD. (Except for *S. Typhi* which has white colonies)

*Escherichia coli* on XLD
FLOW DIAGRAMS

Flow diagram for detection of *Salmonella*

**Non-selective enrichment**
25 g food / faeces in 10% phosphate buffer 37°C, 24 h.

**Selective enrichment**
- 0.1 ml in 10 ml Rappaport-Vassiliadis Soy Broth 37°C, 24 h.
- 1 ml in 10 ml Tetrathionate broth (Müller-Kauffman) 41.5°C, 24 h.

**Isolation**
- XLD with an inoculation loop 37°C, 24 h.
- BLA with an inoculation loop

**Streaking on nutrient agar** 37°C, 24 h.

**Biochemical confirmation** 37°C, 24 h.
- **TSI**
  - Urea broth
- **LDC**
- **ONPG**
- **VP**
- **Indole**

**Serotyping** 37°C, overnight
- **O-antigens**
- **H-antigens**
- **Phase I** 37°C, overnight
- **Phase II**
Flow diagram for the isolation of *Salmonella* from faeces.

Add 25g of faeces to 225ml of buffered peptone water.

- **Transfer 10µl loop / plate**
  - Tetrathionate 10ml
    - Labelled: Tet-faeces #1
  - RVSP. Broth 10ml
    - Labelled: RVSP-faeces #1
  - XLD agar plates.
    - Labelled: XLD-tet-faeces #1
  - BGA agar plates.
    - Labelled: BGA-tet-faeces #1

- **Transfer 10µl loop / plate**
  - Tetrathionate 10ml
    - Labelled: Tet-faeces #2
  - RVSP. Broth 10ml
    - Labelled: RVSP-faeces #2
  - XLD agar plates.
    - Labelled: XLD-tet-faeces #2
  - BGA agar plates.
    - Labelled: BGA-tet-faeces #2
Flow diagram for the isolation of *Salmonella* from food

Add 25g of food to 225ml of buffered peptone water.
**Isolation of *Salmonella* from faeces and food.**

**Morphology on selective agar plates**

### Sample: Faeces #1

<table>
<thead>
<tr>
<th>Colour</th>
<th>Results</th>
<th>Comments</th>
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<tbody>
<tr>
<td>From Tetrathionate: Morphology on BGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology on XLD</td>
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<td></td>
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<tr>
<td>From RVS: Morphology on BGA</td>
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<td>Morphology on XLD</td>
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### Sample: Faeces #2

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

**Morphology on selective agar plates**

### Sample: Food #1

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### Sample: Food #2

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