

HPA STANDARD METHOD

DETECTION OF *SALMONELLA* SPECIES

F 13

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

DETECTION OF *SALMONELLA* SPECIES

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Regional Food, Water and Environmental Coordinators Forum Page 1 of 17

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AMENDMENT PROCEDURE

Controlled document reference	F 13
Controlled document title	Detection of <i>Salmonella</i> species

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
7/ 14/07/08	3	3.1	5	Introduction	Information note added
			6	Safety consideration	Information note added
			10	6.1 Sample preparation and enrichment	Reworded sentence for use of a container capable of closure.
			10	6.2 Selective enrichment	The following sentence is removed 'This selective enrichment stage is omitted for the contents of shell eggs'.
			11	6.4 Procedure for S. Typhi and S. Paratyphi	Paragraph added for the IQC of S. Typhi and S. Paratyphi
			15	Appendix 1	Removed reference to shell egg contents.
			17	References	ISO 7218 added

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DETECTION OF *SALMONELLA* SPECIES

SCOPE OF DOCUMENT

The method described is applicable to the detection of *Salmonella* species in all food types, including milk and dairy products, molluscan shellfish and other fish products, and to its detection in environmental samples such as swabs and dishcloths.

INTRODUCTION

BACKGROUND

The presence of *Salmonella* species in ready-to-eat food is considered significant regardless of the level of contamination². Isolation of *Salmonella* is therefore carried out by enrichment culture of a defined weight or volume of food, which is normally 25 g. For environmental samples such as swabs and dishcloths, the entire sample is usually examined. A pre-enrichment resuscitation stage is incorporated, to allow the recovery of injured cells. The detection of *Salmonella* in food samples can be achieved by a variety of methods, and the methods used can vary in success depending on the type of food being examined.

The method described is based on BS EN ISO 6579:2002³. This uses Rappaport Vassiliadis soya peptone (RVS) broth, which is highly effective for recovery of *Salmonella* from foods with a high level of background contamination. For toxicological reasons it replaces the use of a selenite medium by Muller Kauffmann tetrathionate, novobiocin broth (MKTTn) for the isolation of serotypes of *Salmonella* that are inhibited by the constituents of RVS broth. Although extensive trials have been performed to demonstrate the efficacy of these media for the recovery of *S. Typhi* and *S. Paratyphi*, it is recognised that the combination of these two selective media may not allow recovery of all these strains. This method therefore retains the use of selenite cystine (SC) broth⁴ for samples in which *S. Typhi* and *S. Paratyphi* are specifically sought.

Information note: Only laboratories with appropriate expertise, risk assessments, safety procedures and containment facilities should examine samples for *S. Typhi* and *S. Paratyphi*.

Two isolation media are specified; these are Xylose Lysine Deoxycholate (XLD) agar and brilliant green agar (BGA). If *S. Typhi* or *S. Paratyphi* are specifically sought these isolation media should be supplemented with another selective plating medium that does not contain a high concentration of brilliant green, as this may inhibit the recovery of these strains.

The procedure described in this National Standard Method (NSM) differs slightly from ISO 6579 for dehydrated products as it extends the pre-incubation period to 24h to maximise recovery of stressed organisms. For confirmatory tests it requires the selection of at least five colonies in total, including one from each plating medium, whereas ISO 6579 requires selection of one colony from each plating medium and if they fail to confirm as *Salmonella* a further four colonies per plate are checked (total 20). In addition a different reference strain has been specified in this SOP; an unusual strain has been selected to facilitate detection of cross contamination.

This NSM differs significantly from ISO 6579 in that it gives additional instructions for the detection of *S. Typhi* and *S. Paratyphi*, for preparation of the pre-enrichment suspensions of specific food types including eggs and molluscan shellfish, environmental swabs and dishcloths. It also describes additional procedures to be adopted when investigating public health outbreaks.

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1 PRINCIPLE

The detection of *Salmonella* species in food and environmental samples from the food production environment involves pre-enrichment in a non-selective liquid medium with adjustments as necessary to enhance recovery from certain food types, enrichment in two selective liquid media, subculture onto each of two different selective solid media, and examination for colonies considered to be typical of *Salmonella*. Confirmation of the colonies as *Salmonella* is by means of serological and biochemical tests.

2 DEFINITIONS

For the purposes of this method the following definitions apply:

Salmonella species

Microorganisms which form typical or less typical colonies on solid selective agar media and which display the biochemical and serological characteristics described in this method.

Detection of *Salmonella* species

Determination of the presence or absence of these microorganisms in a defined weight or volume of food or in an environmental swab / dishcloth or other material.

3 SAFETY CONSIDERATIONS

Normal microbiology laboratory precautions apply. In addition, salmonellas are pathogenic to man and therefore isolation and identification must be performed by trained laboratory personnel in a properly equipped laboratory and under the supervision of a qualified microbiologist. Care must be taken in the disposal and sterilisation of all test materials. Procedures involving subculturing from pre - enrichment and enrichment broths and handling of *Salmonella* cultures must be performed in a designated area of the laboratory.

Only laboratories with Containment Level (CL) 3 facilities should examine samples for *S. Typhi* and *S. Paratyphi*. All procedures including preparation of the sample should be performed in a CL3 laboratory by staff trained in the appropriate CL3 procedures. Disposable gloves should be worn during all procedures.

Information note: Requests for the examination of *S. Typhi* and *S. Paratyphi* will occur rarely. In such cases it is accepted that an ongoing quality control programme is not necessary and that appropriate IQC will be performed following sample examination⁵.

Selenium salts are used in the preparation of SC broth. They are toxic if ingested or inhaled and there is a possible risk of teratogenicity. These should not be handled by pregnant laboratory workers.

4 EQUIPMENT

Usual laboratory equipment and in addition:

- Top pan balance capable of weighing to 0.1 g
- Gravimetric diluter (optional)
- pH meter (optional)
- pH indicator strips (range pH 6.5 – 7.5) and sterile inoculation sticks (optional)
- Stomacher
- Incubators: 37° ± 1°C
41.5° ± 1°C
- Waterbath: 37° ± 1°C

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- Stomacher bags (sterile)
- Disposable pipettes/pastettes⁴ capable of delivering 1 mL and 0.1 mL

Note: due to the risks of cross-contamination single use equipment should be used for all subculture procedures. If disposable pipette tips are used these should contain a filter to prevent contamination of the pipettor, and the pipettor regularly decontaminated.

5 CULTURE MEDIA

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.

Buffered peptone water (BPW)

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate or anhydrous disodium hydrogen phosphate	9.0 g 3.5 g
Potassium dihydrogen phosphate	1.5 g
Water	1 L
pH 7.0 ± 0.2 at 25°C	

Rappaport Vassiliadis soya peptone broth (RVS)

Soya peptone	4.5 g
Sodium chloride	7.2 g
Potassium dihydrogen phosphate	1.26 g
Dipotassium hydrogen phosphate	0.18 g
Magnesium chloride (anhydrous)	13.58 g
Malachite green	36 mg
Water	1 L
pH 5.2 ± 0.2 at 25°C	

Muller-Kauffmann tetrathionate novobiocin broth (MKTTn)

Meat extract	4.3 g
Enzymatic digest of casein	8.6 g
Sodium chloride	2.6 g
Calcium carbonate	38.7 g
Sodium thiosulphate pentahydrate	47.8 g
Ox bile	4.78 g
Brilliant green	9.6 mg
Iodine	4.0 g
Potassium iodide	5.0 g
Novobiocin (sodium salt)	0.04 g
Water	1 L
pH (of basal broth) 8.0 ± 0.2 at 25°C	

Selenite cystine broth (SC) (optional)

Tryptone	5.0 g
Lactose	4.0 g
Disodium phosphate	10.0 g
L-Cystine	10 mg
Sodium biselenite	4.0 g
Water	1 L
pH 7.0 ± 0.2 at 25°C	

Xylose lysine desoxycholate agar (XLD)

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Yeast extract	3.0 g
L-lysine hydrochloride	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium desoxycholate	1.0 g
Sodium chloride	5.0 g
Sodium thiosulphate	6.8 g
Ferric ammonium citrate	0.8 g
Phenol red	80 mg
Agar	12.5 g
Water	1 L

pH 7.4 ± 0.2 at 25°C

Brilliant green agar (modified) (BGA)

Meat extract	5.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Disodium hydrogen phosphate	1.0 g
Sodium dihydrogen phosphate	0.6 g
Lactose	10.0 g
Sucrose	10.0 g
Phenol red	0.09 g
Brilliant green	4.7 mg
Agar	12.0 g
Water	1 L

pH 7.0 ± 0.2 at 25°C

Supplementary isolation agar of choice (e.g: Hynes DCA, Rambach agar) (optional)

MacConkey agar (MA)

Bile salts	5.0 g
Enzymatic digest of casein	20.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Neutral red	75 mg
Agar	12.0 g
Water	1 L

pH 7.4 ± 0.2 at 25°C

Nutrient agar (NA)

Meat extract	1.0 g
Enzymatic digest of animal tissues	5.0 g
Agar	12.0 g
Water	1 L

pH 7.0 ± 0.2 at 25°C

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Triple sugar iron agar (TSI) (optional)

Meat extract	3.0 g
Enzymatic digest of casein	20.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Lactose	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Ferric citrate	0.3 g
Phenol red	0.024 g
Agar	12.0 g
Water	1 L
pH 7.4 ± 0.2 at 25°C	

Urea agar (optional)

Enzymatic digest of casein	1.0 g
Glucose	1.0 g
Sodium chloride	5.0 g
Potassium dihydrogen phosphate	5.0 g
Phenol red	0.012 g
40% Urea solution	50 mL
Agar	15.0 g
Water	1 L
pH of base (without urea) 6.8 ± 0.2 at 25°C	

Saline solution

Sodium chloride	8.5 g
Water	1 L

Slopes of nutrient agar, Columbia agar or equivalent

Surfactant eg; Tergitol 7, Sorbitan monooleate (Tween 80); Triton 100 (as required)
10% Potassium sulphite solution; add 12.5 mL to 25 g sample homogenate (as required)
Skimmed milk powder, antibiotic free, 100 g/litre of BPW (as required)
Casein (not acid casein); final concentration 5% (as required)
1M Sodium hydroxide solution
1M Hydrochloric acid solution
Double strength BPW (as required)

Serological reagents for identification of Salmonella species (PSO, PSH etc)

BioMerieux API 20E test kit

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6 SAMPLE PROCESSING

6.1 SAMPLE PREPARATION AND PRE-ENRICHMENT

Prepare the sample using the procedure described in National Standard Method F 2 - Preparation of samples and dilutions⁶. For dairy products follow the procedures described in National Standard Method D 1 - Preparation of samples and decimal dilutions⁷. For molluscan shellfish follow the procedures described in National Standard Method F16 – Enumeration of *Escherichia coli*⁸.

Using sterile instruments and aseptic technique, weigh a representative 25 g sample of each food into a sterile stomacher bag. Using a stomacher, homogenise with nine times that weight or volume of buffered peptone water (BPW). Avoid touching the inside of the bag with the hands. Record the weight of sample and the weight or volume of BPW used. For molluscan shellfish, prepared in a rotary blender, the 1 in 3 slurry may be used by adding 75 g of slurry to 175 mL of BPW.

If the amount of food or dairy product available is less than 25 g or 25 mL maintain the sample:diluent volume at 1:9 (1 in 10). Examination of smaller amounts is also appropriate for low density products such as herbs. Preparation of a 1 in 20 homogenate may be necessary for certain dehydrated products such as dried herbs and vegetables. Ensure that free liquid is present after rehydration.

With some food products the pre-enrichment broth requires additions or adjustments as shown in appendix 2. For highly acidic or alkaline samples the pH of the sample suspension should be checked and adjusted if necessary to 6.8 ± 0.2 ; the pH of the pre-enrichment broth should not drop below 4.5 during incubation. The use of double strength BPW may assist in preventing this.

For very hard products such as dog chews made of hide which cannot be subdivided add sufficient BPW to cover the sample completely.

For environmental swabs, ensure that the swab is completely immersed in BPW, such that an approximate 1 in 10 dilution is achieved. For dishcloths, ensure ample coverage with BPW even after absorption. Mix well by stomaching or vortexing.

Raw shell eggs are usually examined as a batch of 6 eggs or as individual eggs without shell disinfection. Break the contents of the eggs into a tared stomacher bag. It is advisable to use double bags to prevent leakage due to puncture by the shells. Add an equal weight of BPW and homogenise. Transfer the shells to a separate container or bag with closure containing 180 mL BPW and ensure that the shells are covered⁹.

If necessary transfer the homogenate or swab suspension into a container capable of closure (such as a bag or screw topped container)¹⁰. Place in an incubator set at 37°C for 18 ± 2 h. For dehydrated foods the incubation period should be extended to 24 ± 2 h.

6.2 SELECTIVE ENRICHMENT

Transfer 0.1 mL of the pre-enrichment culture to 10 mL of RVS. Place in an incubator set at 41.5°C for 24 ± 3 h. For shell eggs reincubate the RVS broth for a further 24 h.

Transfer 1 mL of the pre-enrichment culture to 10 mL of MKTTn. Place in an incubator set at 37°C for 24 ± 3 h.

If used, transfer 1 mL of the same pre-enrichment culture to 10 mL SC. Place in an incubator at 37°C for 48 ± 3 h.

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6.3 SUBCULTURE TO SELECTIVE AGARS

Following incubation subculture the selective enrichment broths and the pre-enrichment of shell egg contents to XLD and to BGA. Streak out to obtain isolated colonies. Subculture all broths after 24 ± 3 h and again after 48 ± 3 h if the broth was re-incubated. Place in an incubator at 37°C for 24 ± 3 h. Re-incubate negative plates from raw shell eggs for a further 24 ± 3 h.

If *S. Typhi* or *S. Paratyphi* are sought, also subculture to a further isolation medium that does not contain high levels of brilliant green e.g: Hynes DCA.

6.4 PROCEDURE FOR *S.TYPHI* AND *S.PARATYPHI*

Refer to specific safety precautions described in section 3.

Information note: Only laboratories with appropriate expertise, risk assessments, safety procedures and containment facilities should examine samples for *S. Typhi* and *S. Paratyphi*. Requests for the examination of *S.Typhi* and *S. Paratyphi* will occur rarely. In such cases it is accepted that an ongoing quality control programme is not necessary and that appropriate IQC will be performed following sample examination⁵.

In addition to the procedure described above, subculture 1 mL of the incubated BPW to 10 mL of selenite cystine broth (SC). Place in an incubator set at 37°C for 48 ± 3 h. After 24 ± 3 and 48 ± 3 h subculture the SC to XLD, BGA and a further isolation medium that does not contain high levels of brilliant green eg: Hynes DCA. Place in an incubator for 24 ± 3 h.

6.5 PUBLIC HEALTH INVESTIGATIONS

If it is known that *Salmonella* is the causative organism of human infection or the clinical symptoms strongly suggest that this is the cause of illness it is advisable to perform the following additional procedures:

Subculture the pre-enrichment suspension before incubation, and the pre-enrichment culture after incubation directly to selective agar media.

Retain the incubated pre-enrichment culture under refrigeration until investigations are complete.

Re-incubate the RVS and MKTTn broths for a further 24 ± 3 h and then subculture again to selective agars.

Retain the RVS and MKTTn broths under refrigeration until investigations are complete.

Re-incubate negative plates for a further 24 ± 3 h.

6.6 RECOGNITION OF COLONIES

After 24 h examine the selective agar plates for typical and less typical colonies of *Salmonella*. On XLD, *Salmonella* ferment xylose, normally decarboxylate lysine, and produce hydrogen sulphide. Characteristic colonies are red with black centres. Isolated colonies may appear yellow with black centres. *Salmonella* species that produce little or no hydrogen sulphide eg: *S. Typhi*, *S. Senftenberg*, *S. Pullorum* grow as red colonies with or without black centres. Red colonies may also be produced by some strains of *Proteus* species and *Pseudomonas* species. Strains of *S. Paratyphi* A do not decarboxylate lysine and so appear as yellow colonies usually with a black centre. Lactose fermenting strains may also appear yellow with or without black centres.

Note: All strains of *S. Typhi* and *S. Paratyphi* other than *S. Paratyphi* A are lysine positive. Production of black colonies due to hydrogen sulphide on XLD is variable. *S. Typhi* may not grow on XLD.

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On BGA, *Salmonella* species do not normally ferment sucrose or lactose and produce red colonies surrounded by a bright red medium. Red colonies may also be produced by some strains of *Proteus* species and *Pseudomonas* species. Strains of *S. Typhi* and *S. Paratyphi* may not grow on BGA.

6.7 CONFIRMATORY TESTS

Typical or less typical suspect colonies of *Salmonella* from each plate must be subjected to serological and biochemical confirmation. The extent of testing may be reduced once the presence of *Salmonella* is established (see below).

Select at least five suspect *Salmonella* colonies including one from each of the selective agar plate/broth combinations; subculture to MA and NA to obtain isolated colonies. Place in an incubator at 37°C for 21 ± 3 h.

If isolated colonies are available, also screen biochemically using TSI agar slopes and urea agar or a commercial biochemical screening kit. After inoculation of these media a purity check should be performed by inoculation of MA and NA. Incubate all media at 37°C for 21 ± 3 h. Check the agar plates at the end of incubation for purity.

Serological confirmation

Subculture non-lactose fermenting colonies from the above media to slopes of NA or equivalent dispensed in universal containers. Ensure that some water of condensation is present at the base of the slope; if none is present then add a few drops of sterile water. Inoculate the colony into the water of condensation and streak up the slope. Incubate at 37°C for at least 5 ± 1 h, or overnight if necessary.

Using the growth from the NA slope, prepare three saline suspensions on a slide using a loopful of saline and growth from: -

the slope for 'O' antigens

the water of condensation at the bottom of the slope for 'H' antigens

a mixture from slope and condensate for autoagglutination. If autoagglutination occurs proceed to biochemical confirmation.

Add a loopful of polyvalent 'O' and polyvalent 'H' antisera to two separate saline suspensions and rock the slide gently for 30 seconds. If agglutination occurs with the polyvalent antisera but not with the saline the reactions are considered to be positive.

Identify to at least *Salmonella* group level by agglutination with specific 'O' antisera. If possible also identify at least one 'H' antigen. Record the results.

The first isolate must be serologically typed as comprehensively as possible, determining the 'O' and 'H' antigens. All subsequent isolates must be serotyped to determine the 'O' antigen and one 'H' antigen only. If the slide agglutination reactions indicate that subsequent isolates differ serologically from the first isolate, then further serological and biochemical testing must be performed.

Some *Salmonella* may give agglutination with 'H' antisera but not with 'O' antisera. This could be due to the presence of antigens not included in the polyvalent 'O' antiserum or to the masking of 'O' antigens by capsular antigens. Such isolates must either be tested with individual 'O' antisera or, Vi antiserum if *S. Typhi* is suspected.

Biochemical confirmation

If biochemical screening has been performed directly from the isolation plates, examine the purity plates to check for purity. Then examine the corresponding TSI and urea slopes for the pure strains. Strains of *Salmonella* typically produce an acid (yellow) butt with gas bubbles

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and an alkaline (cerise/deep pink) slope, with blackening due to hydrogen sulphide production. This blackening may mask the acid production in the butt, but is occasionally absent. Strains of *Salmonella* do not produce urease (rare exceptions) so no change in colour is seen in the urea agar.

If biochemical screening has not been done or if the screening results suggest the presence of a *Salmonella*, select discrete colonies from the NA purity plates or growth from a NA slope that has been confirmed to be pure. Identify at least one isolate giving biochemical and/or serological reactions consistent with *Salmonella* or those giving equivocal serological results using an API 20E biochemical identification gallery. Follow the manufacturer's instructions. Acceptable profiles are good, very good or excellent identification with a percentage of identification $\geq 90\%$ and a T index ≥ 0.25 . If a doubtful or unacceptable profile is obtained recheck the purity; if pure and the presence of *Salmonella* remains a possibility, send the strain to the reference laboratory for further identification.

If biochemical results exclude the presence of *Salmonella* and the strain is pure no further action is required.

Note: If more than one serotype is present then a representative of each serotype must also be identified biochemically.

Public Health Investigations

In some instances a rapid response may be required. Provisional recognition of *Salmonella* can be obtained by performing agglutination with polyvalent 'O' and 'H' antisera on discrete colonies obtained on the primary isolation media. Alternatively NA slopes may be inoculated directly from the selective agar but a purity check on MA must also be inoculated after inoculation of the slopes.

Control cultures

Positive control: *Salmonella* Nottingham (16:d:enz₁₅) NCTC 7832

Negative control: *Escherichia coli* NCTC 9001

7 REPORTING OF RESULTS

If *Salmonella* species are not recovered report as follows:

Salmonella species not detected in 25 g, 25 mL or swab

The actual weight or volume of sample examined must be reported, for example, 10 g or mL, 25 g or mL, 100 g or mL, unless the product has been examined without reference to weight (shell eggs, hide dog chews).

If a *Salmonella* is recovered from a ready to eat food the laboratory should review its procedures in accordance with the advice given in QSOP 37 – Procedure for dealing with presumptive pathogens isolated from ready-to-eat foods¹¹ before reporting the result.

If the presence of *Salmonella* species has been confirmed by biochemical and serological testing and the lead microbiologist is satisfied with the procedural review, report as follows:

Salmonella species detected in 25 g, 25 mL, or sample

A further report from the reference laboratory may be necessary giving details of the serotype or phage type.

If the biochemical or serological results are inconclusive but the presence of *Salmonella* is still considered to be a strong possibility the report may be reported as a presumptive detection with the confirmation from the Reference Laboratory to follow.

DETECTION OF SALMONELLA SPECIES

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Note: It is a statutory requirement to report isolations of *Salmonella* from live animals and poultry, animal by-products not intended for human consumption, and animal/poultry feedstuffs and ingredients to the Veterinary Laboratories Agency (VLA) and to send isolates derived from the animal feed to the regional VLA laboratory applicable to the production site of the feed^{12,13}.

8 REFERENCE FACILITIES

All *Salmonella* isolates from ready to eat foods, shell eggs and food poisoning samples must be sent to the Laboratory of Enteric Pathogens (LEP), HPA Centre for Infections for confirmation and full identification in terms of biochemistry, serology and, if necessary, phage type¹¹.

9 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method has been developed, reviewed and revised by the Food and Dairy Working Group for National Standard Methods (http://www.hpa-standardmethods.org.uk/wg_food_dairy_group.asp). The contributions of many individuals in Food, Water and Environmental laboratories, reference laboratories and specialist organisations who have provided information and comment during the development of this document are acknowledged.

The National Standard Methods are issued by Standards Unit, Evaluations and Standards Laboratory, Centre for Infections, Health Protection Agency, London.

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APPENDIX 1: FLOWCHART SHOWING THE PROCESS FOR THE DETECTION OF *SALMONELLA* SPECIES

Weigh or measure 25 g/mL of sample, add 225 mL buffered peptone water (with additions if required)

If sample is a swab, dish cloth or hide dog chew completely immerse in buffered peptone water to approximate 1 in 10



Homogenise by stomaching

If high or low pH product, check pH of suspension and adjust as necessary



Incubate at 37°C for 18 ± 2 h
(24 h for dehydrated foods)



Inoculate 0.1 mL into 10mL RVS broth



Inoculate 1 mL into 10 mL of MKTTn broth



Inoculate 1 mL into 10 mL of SC broth
(If *S. Typhi* or *S. Paratyphi* sought)



Incubate at 41.5°C for 24 ± 3 h



Incubate at 37°C for 24 ± 3 h



Incubate at 37°C for 24 ± 3 h



Subculture onto XLD and BGA



Subculture onto XLD and BGA



Subculture onto XLD and BGA and a third medium of choice

Re-incubate broth at 41.5°C for 24h if outbreak or shell egg samples and subculture again to XLD and BGA

Re-incubate broth at 37°C for 24h if outbreak or shell egg samples and subculture again to XLD and BGA

Re-incubate broth at 37°C for 24h and subculture again to XLD, BGA and third medium



Incubate plates at 37°C for 24 ± 3 h

Re-incubate negative plates for further 24 ± 3 h if outbreak or shell egg samples



If present subculture five typical *Salmonella* colonies including at least one typical colony from each plate to MA and NA



Incubate at 37°C for 21 ± 3 h



Eliminate non-*Salmonella* colonies and identify *Salmonellas* using serology and biochemistry



Send to Laboratory for Enteric Pathogens for typing

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APPENDIX 2: TABLE OF ADDITIONS/ADJUSTMENTS TO SALMONELLA ENRICHMENT BROTHS

Product	Additions/adjustments	Purpose
High fat foods eg cheese	Add surfactant (eg tergitol 7; final concentration of 0.22%, Triton 100, Tween 80; final concentration 1.0%)	Aids food dispersion
Onion & garlic	Add potassium sulphite to give a final concentration of 0.5%	Reduces natural bactericidal properties
Cocoa powder & chocolate confectionery	Add skimmed milk powder to give a final concentration of 10% or casein (not acid casein) to give a final concentration of 5%	Reduces bactericidal properties
High salt/sugar	Increase sample to broth ratio to obtain final concentration below 2%	Maintains salt or sugar concentration below 2%
Oregano, cinnamon, cloves, Allspice	Increase sample to broth ratio eg: 1/100 for allspice, cinnamon, oregano 1/1000 for cloves	Reduces inhibitory properties
High pH foods (eg egg albumen) and low pH foods eg cheeses, mayonnaise, vinegar based marinades, fruits	Adjust pH to 6.8 ± 0.2 prior to incubation	Neutralises the antibacterial effect of acid and alkali
Some low pH foods eg: freeze dried berry fruits	Suspend in double strength BPW	Prevents pH dropping below 4.5 during incubation

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