INTERNATIONAL STANDARD

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Water quality — Detection of *Salmonella* species

Qualité de l'eau — Recherche de Salmonella

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 6340 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*.

Annexes A and B of this International Standard are for information only.

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Introduction

Salmonella species are bacteria which are widely distributed all over the world. They are usually classified as pathogens, although their virulence and pathogenesis vary widely. The natural hosts of *Salmonella* species include humans, agricultural and domestic livestock and wild animals including birds. Humans and animals may excrete these bacteria while carrying them asymptomatically, as well as during disease. It is therefore impossible to eliminate them from the environment. Due to the severe diseases which can follow the infection of humans, the transmission of *Salmonella* species via different vehicles has to be minimized.

Since water is one of the vehicles, the presence or absence of *Salmonella* species should be monitored in water. *Salmonella* species may be present in all types of domestic and agricultural sewage, fresh waters, including ground and drinking waters, and also sea water.

The detection of *Salmonella* in water usually requires a concentration step. Since cells of *Salmonella* species may be injured in the aqueous environment, their detection in water usually requires a pre-enrichment step. The procedure described in this International Standard consists of regular enrichment(s), selection and confirmation steps.

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Water quality — Detection of *Salmonella* species

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *Salmonella* species are undertaken in properly equipped laboratories, under the control of skilled microbiologists only, and that great care is taken in the disposal of all incubated materials.

1 Scope

This International Standard specifies a method for the detection of *Salmonella* species in water samples for monitoring purposes. In special epidemiological situations, other media may also be required.

The method can be applied to all kinds of water, except raw sewage.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods.*

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes.*

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques.*

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples.*

ISO 6579:1993, *Microbiology — General guidance on methods for the detection of Salmonella.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 *Salmonella* species: Gram-negative, oxidase-negative, facultatively anaerobic, non-sporeforming, rod-shaped bacteria which generally form colonies of 2 mm to 4 mm in diameter on solid selective media. They form typical colonies on solid selective media and display the biochemical and serological characteristics described when tests are carried out in accordance with this International Standard.

3.2 detection of *Salmonella* organisms: Determination of the presence of these bacteria in a particular volume, when tests are carried out in accordance with this International Standard.

4 Principle

The detection of *Salmonella* species requires four successive stages.

4.1 Pre-enrichment

Pre-enrichment is necessary to enable injured cells to grow. If necessary, samples can be concentrated using membrane filtration. The membrane filter with cells, or a known volume of sample or its dilution, is transferred to non-selective broth (buffered peptone water) for incubation at the optimal temperature for mesophilic bacteria.

4.2 Enrichment in selective liquid medium

A selective enrichment step is necessary to increase the proportion of *Salmonella* species in relation to background flora. For this purpose, inoculum from pre-enrichment broth is transferred to malachite green/magnesium chloride (modified Rappaport-Vassiliadis) medium which is incubated at an elevated temperature to increase its selectivity.

NOTE 1 For the detection of *Salmonella typhi*, which is usually not important for water quality monitoring but may be required under special circumstances, selenite cystine medium (also available as dehydrated complete medium from different manufacturers) can be used for incubating the cultures at 36 °C ± 2 °C for up to 24 h. In certain special epidemiological situations, addition of other media may be necessary.

4.3 Selection on agar media

Solid selective media are used after the liquid enrichment steps for the detection and isolation of *Salmonella* species. In order to increase the probability of detecting *Salmonella* organisms, at least two different media are inoculated from selective enrichment cultures:

— brilliant green/phenol red lactose agar;

— xylose lysine deoxycholate agar;

— bismuth sulfite agar (optional).

4.4 Confirmation

The occurrence of typical colonies of *Salmonella* species on selective agar media is not sufficient evidence for the presence of *Salmonella* species. Therefore, it is necessary to subculture presumptive *Salmonella* colonies on different media for biochemical and serological confirmation (see table 1).

NOTE 2 Commercially available identification kits suitable for the identification of *Salmonella* species can be used instead of the tests listed in table 1, provided that they are used according to the manufacturer's instructions and on condition that they can be considered at least as reliable as the tests listed in table 1.

5 Culture media and confirmation media

Use reagents of analytical quality for the preparation of culture media, unless otherwise specified. Prepare media using glass-distilled water, or water of equivalent quality, complying with grade 3 of ISO 3696.

If commercially available dehydrated media are used, prepare them according to the manufacturer's instructions and add selective agents as supplements to give the specified concentrations.

All pH values given in this International Standard are for media after sterilization; for pH correction, use sodium hydroxide or hydrochloric acid at concentrations of 1 mol/l each.

5.1 Culture media

5.1.1 Pre-enrichment medium: buffered peptone water

5.1.1.1 Composition

	Single strength	Double strength
Peptone	10 g	20 g
Sodium chloride (NaCl)	5 g	10 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9 g	18 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g	3 g
Water	to 1 000 ml	to 1 000 ml

5.1.1.2 Preparation

Dissolve all the constituents in water by heating gently, but do not boil the solution.

Adjust the pH to 7,2 ± 0,1, with sodium hydroxide solution or hydrochloric acid.

Dispense the medium into culture bottles/tubes.

Sterilize the medium in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Store in a refrigerator for up to 3 months.

5.1.2 Enrichment medium: malachite green/magnesium chloride (modified Rappaport-Vassiliadis medium)

5.1.2.1 Composition

Basic medium	
Peptone, enzymatic digest of animal tissue	4 g
Peptone, from soybeans	1 g
Sodium chloride (NaCl)	8 g
Dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$)	0,4 g
Potassium dihydrogen phosphate (KH_2PO_4)	0,6 g
Water	to 1 000 ml

Supplement 1 [1]	
Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)	31,7 g
Water	to 100 ml

1) As this salt is very hygroscopic, it is advisable either to store it in a dessicator or to dissolve the entire contents of a newly opened container of magnesium chloride in such a way that the mass concentration of magnesium chloride hexahydrate is 28,6 g/l in the final medium. The magnesium chloride solution can be stored for a long time in a sealed container.

Supplement 2	
Malachite green oxalate	0,4 g
Water	to 100 ml

5.1.2.2 Preparation

Dissolve all the constituents of the basic medium in water by heating gently, but do not boil the solution.

Add the prepared magnesium chloride solution (supplement 1) and 10 ml of the malachite green solution (supplement 2) to the basic medium.

Adjust the pH to 5,2 ± 0,1, with sodium hydroxide solution or hydrochloric acid.

Dispense about 10 ml of the medium into each culture tube.

Sterilize the medium in the autoclave (6.1.2) at 115 °C ± 1 °C for 15 min.

5.1.3 Optional enrichment medium: selenite cystine

5.1.3.1 Composition

Casein-peptone	5 g
L-Cystine	0,01 g
Lactose	4 g
Disodium hydrogen phosphate (Na_2HPO_4)	10 g
Sodium hydrogen selenite ($NaHSeO_3$)	4 g
Water	to 1 000 ml

5.1.3.2 Preparation

Dissolve all the constituents in water by heating gently, but do not boil the solution.

CAUTION — Do not sterilize the medium in the autoclave. Apply sterile filtration instead, and do not use the medium if red sediments appear.

Adjust the pH to 7,0 ± 0,2.

WARNING — Inhalation of sodium hydrogen selenite dust and direct contact with the skin is very dangerous. The dust irritates the eyes, skin and mucous membranes and can penetrate skin both as a powder and as a solution. It has long-term health effects and may be carcinogenic. Reaction with acids liberates gaseous hydrogen selenide, which is very dangerous if inhaled and irritate the eyes and mucous membranes. Sodium hydrogen selenite and its solution must be handled under a hood using gloves and, if required, a respirator mask should be used. Contact with acids must be avoided. Store in tightly closed containers in a well-ventilated area that is dry and separated from acids.

5.1.4 Selective solid media

5.1.4.1 Brilliant green/phenol red lactose agar (according to Edel and Kampelmacher)

5.1.4.1.1 Composition

Basic medium	
Meat extract powder	5 g
Peptone, enzymatic digest of animal tissue	5 g
Disodium hydrogen phosphate (Na_2HPO_4)	1 g
Sodium dihydrogen phosphate (NaH_2PO_4)	0,6 g
Agar	about 15 g
Water	to 900 ml

Supplement 1	
Lactose	10 g
Sucrose	10 g
Phenol red	0,09 g
Water	to 100 ml

Supplement 2	
Brilliant green	0,5 g
Water	to 100 ml

5.1.4.1.2 Preparation

Dissolve all the constituents of the basic medium in water, and sterilize in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Prepare supplement 1 by dissolving the constituents in sterile water. Heat the solution in a water bath (6.2) at 70 °C for 20 min. Cool it to 55 °C ± 1 °C and use it immediately.

Prepare supplement 2 by dissolving the brilliant green in the water. Store the solution for at least 1 day in the dark to allow autosterilization to occur.

Add the prepared sugar/phenol red solution (supplement 1) and 1 ml of the brilliant green solution (supplement 2) to the agar before distribution into Petri dishes (6.7); ensure that the final pH is 7,0 ± 0,1. Immediately before use, dry the agar plates until the surface of the agar is dry. Use freshly prepared plates.

5.1.4.2 Xylose lysine deoxycholate agar

5.1.4.2.1 Composition

Basic medium	
D(+)-Xylose	3,5 g
L(+)-Lysine	5 g
Sodium deoxycholate	2,5 g
Yeast extract	3 g
Saccharose	7,5 g
Lactose	7,5 g
Sodium chloride (NaCl)	5 g
Sodium thiosulfate ($Na_2S_2O_3$)	6,8 g
Iron(III) ammonium citrate	0,8 g
Agar	about 13 g
Water	to 1 000 ml

Supplement	
Phenol red	0,4 g
Water	to 100 ml

5.1.4.2.2 Preparation

Dissolve all the constituents, including 20 ml of the phenol red solution (supplement), by heating to bring to the boil.

Adjust the pH to 7,4 ± 0,1.

CAUTION — To avoid overheating, do not prepare portions larger than 1 litre at a time. Do not sterilize the medium in the autoclave. After preparation, transfer to a water bath at 50 °C and pour into Petri dishes (6.7) as soon as the medium has cooled.

5.1.4.3 Bismuth sulfite agar (according to Wilson and Blair)

5.1.4.3.1 Composition

Basic medium	
Meat extract	5 g
Peptone, enzymatic digest of animal tissue	10 g
D(+)-Glucose	5 g
Disodium hydrogen phosphate (Na_2HPO_4)	4 g
Iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$)	0,3 g
Bismuth sulfite [$Bi_2(SO_3)_3$]	8g
Agar	about 15 g
Water	to 1 000 ml

Supplement	
Brilliant green	0,5 g
Water	to 100 ml

5.1.4.3.2 Preparation

Dissolve all the constituents, including 5 ml of the brilliant green solution (supplement), by heating. Do not sterilize the medium in the autoclave.

Adjust the pH to 7,6 ± 0,1 and pour about 20 ml of the dissolved, but cloudy medium into each Petri dish (6.7). Ensure that the agar is pale brownish or reddish yellow (fallow) or greenish. If the colour is brown, do not use the medium.

5.2 Confirmation media

5.2.1 Nutrient agar

5.2.1.1 Composition

Meat extract	3 g
Peptone, enzymatic digest of animal tissue	5 g
Agar	about 15 g
Water	to 1 000 ml
Sodium chloride (NaCl) (optional)	5 g

5.2.1.2 Preparation

Dissolve all the constituents by boiling.

Adjust the pH to 7,0 ± 0,1.

Sterilize the medium in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Dispense into Petri dishes (6.7).

5.2.2 **Iron/two-sugar agar** (according to Kligler)

5.2.2.1 Composition

Basic medium	
Meat extract	3 g
Yeast extract	3 g
Peptone, enzymatic digest of animal tissue	20 g
Lactose	10 g
D(+)-Glucose	1 g
Iron(III) citrate	0,5 g
Sodium chloride (NaCl)	5 g
Sodium thiosulfate ($Na_2S_2O_3$)	0,3 g
Agar	about 12 g
Water	to 1 000 ml

Supplement	
Phenol red	0,4 g
Water	to 100 ml

5.2.2.2 Preparation

Dissolve all the constituents, including 6 ml of the phenol red solution (supplement), by heating.

Adjust the pH to a final value of 7,4 ± 0,1.

Sterilize the medium in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Pour about 6 ml of the medium into each tube.

Allow the medium to set in a sloping position to give a butt of length about 2,5 cm.

5.2.3 **Urea agar** (according to Christensen)

5.2.3.1 Composition

Basic medium	
Peptone, enzymatic digest of animal tissue	1 g
D(+)-Glucose	1 g
Sodium chloride (NaCl)	5 g
Potassium dihydrogen phosphate (KH_2PO_4)	2 g
Agar	about 15 g
Water	to 1 000 ml

Supplement 1	
Phenol red	0,4 g
Water	to 100 ml

Supplement 2	
Urea	40 g
Water	to 100 ml

5.2.3.2 Preparation

Dissolve all the constituents of the basic medium and 3 ml of the phenol red solution (supplement 1), by heating if necessary.

Sterilize the medium in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Allow the agar to cool to about 50 °C.

Sterilize the urea solution (supplement 2) by filtration and add 50 ml of this solution under aseptic conditions to 950 ml of the agar (basic medium and supplement 1).

Dispense about 6 ml of the urea agar medium into each tube.

Ensure that the final pH is 6,8 ± 0,1.

Allow to set in a sloping position.

5.2.4 L-Lysine decarboxylase medium (according to *Falkow*)

5.2.4.1 Composition

Basic medium	
L-Lysine monohydrochloride	5 g
Yeast extract	3 g
Peptone from meat	5 g
D(+)-Glucose	1 g
Water	to 1 000 ml

Supplement	
Bromocresol purple	0,5 g
Water	to 100 ml

5.2.4.2 Preparation

Dissolve all the constituents, including 3 ml of the bromocresol purple solution (supplement), by boiling.

Adjust the pH to 6,8 ± 0,1.

Dispense 5 ml of the medium into each tube.

Sterilize the medium in the autoclave (6.1.2) at 121 °C ± 1 °C for 10 min.

5.3 Serological confirmation

5.3.1 Saline solution

5.3.1.1 Composition

Sodium chloride (NaCl)	0,85 g
Water	to 100 ml

5.3.1.2 Preparation

Dissolve the sodium chloride in the water.

Adjust the pH to 7,0 ± 0,1.

Sterilize in the autoclave (6.1.2) at 121 °C ± 1 °C for 15 min.

Dispense smaller volumes into tubes.

5.3.2 Sera

Agglutinating sera are available either commercially or from specialized laboratories. Anti-O sera are always required but often anti-Vi and anti-H sera are used as well (see ISO 6579). Serological tests should be carried out in laboratories with a sufficient internal quality control and experience of serology, to enable selection of relevant sera and controls.

6 Apparatus

Usual microbiological laboratory equipment and the following.

6.1 Apparatus for sterilization

6.1.1 Oven (dry sterilizer), for glassware, capable of being maintained at 170 °C to 175 °C for 1 h.

6.1.2 Autoclave (wet sterilizer), for equipment, culture media and reagents, capable of being maintained at 121 °C ± 1 °C and at 115 °C ± 1 °C.

6.2 Water bath, capable of being maintained at 70 °C ± 1 °C.

6.3 Incubator, capable of being maintained at 36 °C ± 2 °C.

6.4 Water bath or incubator, capable of being maintained at 42 °C ± 0,5 °C.

6.5 pH-meter, having an accuracy of calibration of 0,1 pH unit at 25 °C.

6.6 Loops, of diameter about 3 mm.

6.7 Petri dishes, sterile, with a diameter of either 90 mm or 100 mm.

6.8 Membrane filter, sterile, usually about 47 mm or 50 mm in diameter and with filtration characteristics equivalent to a rated nominal pore diameter of 0,45 µm.

7 Sampling

Take samples in accordance with ISO 5667-1, ISO 5667-2 and ISO 5667-3. Keep them cool at 2 °C to 5 °C and protect them from light. The maximum suitable preservation time before analysis is 24 h. Start the analysis as soon as possible within 24 h.

NOTE 3 The volume of the sample depends on the expected density of *Salmonella* cells, on the character of the water and on legal requirements for water quality standards.

8 Procedure

8.1 Pre-enrichment

For water samples exceeding 10 ml in volume, add the sample to the same volume of buffered peptone water (double strength) (5.1.1.1)[1] or filter through a sterile membrane filter (6.8) and place the filter in 50 ml of buffered peptone water (single strength) (5.1.1.1). Filtering aids can be used when needed.

For sample volumes of 10 ml or less, use a minimum of 50 ml of buffered peptone water (single strength) or at least 10 times the volume of the sample.

Incubate at 36 °C ± 2 °C for 16 h to 20 h, and proceed to 8.2.

8.2 Enrichment in selective liquid media

Transfer 0,1 ml of the pre-enrichment culture (8.1) to 10 ml, or 1 ml to 100 ml, of malachite green/magnesium chloride medium (5.1.2) and incubate in a water bath (6.4) at 42 °C ± 0,5 °C for 18 h to 24 h. (If an incubator other than a water bath is used, prewarm the media at this temperature before inoculation.) The larger volume of inoculum might increase the probability of detecting *Salmonella* organisms. In certain situations, the use of selenite cystine medium (5.1.3) in addition to malachite green/magnesium chloride medium is recommended (see note 1 to 4.2).

Proceed to 8.3 and 8.4.

NOTE 4 In order to detect slow-growing *Salmonella* species, it is recommended to reinoculate solid media (see 8.3) after continued incubation of selective liquid media for another 24 h.

8.3 Selection on agar media

Plate a loopful (see 6.6) of enrichment medium (see 8.2) onto

brilliant green/phenol red lactose agar (5.1.4.1);

xylose lysine deoxychlolate agar (5.1.4.2);

and (optional)

bismuth sulfite agar (5.1.4.3).

Place in an incubator (6.3) at 36 °C ± 2 °C for 24 h (48 h for bismuth sulfite agar).

8.4 Confirmation

For confirmation, take all (or at least five of) the distinct typical *Salmonella* colonies from each positive agar medium (see 8.3):

— colonies on brilliant green/phenol red lactose agar (5.1.4.1) which are red or slightly pink-white and opaque with red surroundings;

— colonies on xylose lysine deoxycholate agar (5.1.4.2) which are colourless (but appear red), usually with a black centre;

— black colonies on bismuth sulfite agar (5.1.4.3), usually surrounded by a metallic sheen.

Plate out the selected colonies onto the surface of predried nutrient agar plates (5.2.1) in a manner which will allow well-isolated colonies to develop.

Place these plates in an incubator (6.3) at 36 °C ± 2 °C for 18 h to 24 h.

Use single isolated colonies only. If the plating fails to produce distinct colonies, repeat in a manner which ensures that single discrete colonies are produced.

8.4.1 Biochemical confirmation (basic procedures)

Table 1 lists the biochemical reactions of typical *Salmonella* species. At least 90 % of *Salmonella* strains produce the indicated reaction.

Table 1 — Basic biochemical reactions

Marker	Reaction	Medium
Lactose	–	Iron/two-sugar agar
Glucose	+	Iron/two-sugar agar
Hydrogen sulfide	+	Iron/two-sugar agar
Urea	–	Urea agar
Lysine decarboxylase	+	Lysine decarboxylase medium

1) Mineral waters with high salt contents and sea water should not be investigated by this type of pre-enrichment due to their high salt concentrations.

Use cells from single colonies on nutrient agar of the culture to be confirmed to inoculate the biochemical test media (see 8.4). Repeat for each culture to be identified.

8.4.1.1 Lactose/glucose fermentation and hydrogen sulfide formation

Streak a colony and stab the butt into iron/two-sugar agar (5.2.2).

Place in an incubator (6.3) at 36 °C ± 2 °C for 24 h.

Typical *Salmonella* cultures show red slants with gas formation and yellows butts with blackening of the agar.

8.4.1.2 Urea degradation

Inoculate a colony into a tube of urea agar (see 5.2.3).

Place in an incubator (6.3) at 36 °C ± 2 °C for 24 h.

Typical *Salmonella* cultures show a negative reaction (no rose-pink colour followed by deep cerise).

8.4.1.3 L-Lysine decarboxylase medium

Inoculate a colony just below the surface of the liquid L-lysine decarboxylase medium (5.2.4). Overlay the medium afterwards with sterile liquid paraffin or oil.

Place in an incubator (6.3) at 36 °C ± 2 °C for 24 h.

Typical *Salmonella* cultures show a purple colour.

8.4.2 Serological confirmation

Use slide agglutination with the appropriate antisera (see ISO 6579) to detect *Salmonella* antigens from discrete single colonies on nutrient agar after auto-agglutinable strains have been eliminated.

8.4.2.1 Elimination of auto-agglutinable strains

Place one drop of the saline solution (5.3.1) onto a thoroughly cleaned glass slide. Disperse this drop in the smooth colony to be tested, to obtain a uniform suspension. If the colony is not smooth, proceed as for auto-agglutinable strains.

Rock the slide gently for 30 s to 60 s.

Observe the result against a dark background, preferably with the aid of a magnifying glass.

If the suspension forms large clumps, regard the strain as auto-agglutinable. Do not use it for further serological tests. It is better to select another colony for serology, but, if desired, the auto-agglutinable colony can be investigated biochemically for confirmation as a *Salmonella* species.

8.4.2.2 Examination for O-antigens

Using one colony recognized as non-auto-agglutinable, proceed according to 8.4.2.1, using 1 drop of the anti-O serum (5.3.2) instead of the saline solution.

Use the polyvalent and monovalent sera one after the other.

Regard agglutination with antiserum as a positive reaction.

8.4.2.3 Definitive confirmation

Send strains which are considered to be *Salmonella* species, or which may be *Salmonella* species, to a reference laboratory for definitive typing.

9 Expression of results

In accordance with this procedure, indicate the presence or absence of *Salmonella* in the test portion examined, the results for biochemical reactions listed in table 1, serological test results and the procedures in 8.4.2.

10 Test report

The test report shall include the following information:

a) a reference to this International Standard;

b) all details necessary for complete identification of the sample;

c) the result, expressed as the presence or absence of *Salmonella* in a test portion of x ml of water;

d) the number of strains isolated from selective solid media (see 8.4), test results of biochemical confirmation (8.4.1) and serological confirmation (8.4.2) indicating the species/serotypes observed.

11 Quality assurance

Performance characteristics for the detection of different *Salmonella* species and subspecies cannot be given because of variations between species and subspecies. However, in the selective enrichment step (8.2) the magnesium chloride concentration and incubation temperature have been optimized to yield

good recovery without losing selectivity (see [2] in annex B).

To check the ability of the laboratory to detect *Salmonella*, introduce reference samples into control flasks of the pre-enrichment medium (5.1.1) and proceed with these control flasks according to 8.1 to 8.4. For example, about ten *Salmonella* cells in the inoculum with the background of 10^8 cells of *Escherichia coli* shall be detected.

Annex A
(informative)

Additional microbiological information relevant to examination of water samples for *Salmonella* organisms

For routine water examination purposes, the *Salmonella* organisms may be described generally in microbiological, although not in taxonomic terms as follows.

The *Salmonella* genus belongs to the family *Enterobacteriaceae*. *Salmonella* organisms are Gram-negative, non-sporing, oxidase-negative, rod-shaped bacteria, which are capable of aerobic and facultatively anaerobic growth. They are able to produce hydrogen sulfide, L-lysine decarboxylase and to ferment glucose, but usually not lactose. They are not able to produce urease.

Annex B
(informative)

Bibliography

[1] ISO 8199:1988, *Water quality — General guide to the enumeration of micro-organisms by culture.*

[2] PETERZ, M., WIBERG, C. and NORBERG, P. The effect of incubation temperature and magnesium chloride concentration on growth of *Salmonella* in home-made and in commercially available dehydrated Rappaport-Vassiliadis broths. *J. Appl. Bact.* **66** (1989), pp. 523-528.

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