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Soil improvers and growing media — Detection of *Salmonella* spp.

Einführendes Element — Haupt-Element — Ergänzendes Element

Élément introductif — Élément central — Élément complémentaire

ICS:

CCMC will prepare and attach the official title page.

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European foreword

This document (prEN XXXX:XXXX) has been prepared by Technical Committee CEN/TC 223 "Soil improvers and growing media", the secretariat of which is held by NEN.

This document is a working document.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

This document shall be used in combination with EN 13040-2, *Soil improvers and growing media – Sample preparation – Part 2: Sample preparation for microbiological examination.*

Introduction

This document describes a method for the detection of *Salmonella* spp. in soil improvers and growing media.

This methodology has been developed to detect *Salmonella* spp. in soil improvers and growing media in order to be able to control certain imposed hygienic requirements. The method is based on EN ISO 6579-1 [4,5].

Salmonella spp. are rod-shaped Gram negative bacteria of faecal/intestinal origin within the family of Enterobacteriaceae. Consequently, it can be used as an indicator of faecal contamination and as a parameter to evaluate the sanitation process during the manufacturing process of soil improvers or growing media. The presence or absence of *Salmonella* spp. does not reflect the presence or absence of other pathogens in the examined product.

Some components of soil improvers (e.g. bog peat, *Sphagnum*) have a very low pH value, others (e.g. compost) can be slightly alkaline and have a negative impact on the survivability of microorganisms during the preparation of the initial suspension. Therefore, it may be necessary to measure the pH of the initial suspension.

Some considerations have to be taken into account during sample preparation, for this see draft EN 13040-2 [2].

1 Scope

This document specifies a method for the detection of *Salmonella* spp. in soil improvers and growing media. This document applies to material in solid form (including pre-shaped growing media) and liquid form.

The detection of *Salmonella* requires four successive steps: a pre-enrichment, a second enrichment, an isolation on a chromogenic agar, and if positive a confirmation with a serological test (and if required, a selective media).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12579[1], *Soil improvers and growing media – Sampling*

EN 13040-2[2], *Soil improvers and growing media – Part 2: Sample preparation for microbiological examination*

WI 00223137: –[3], *Soil improvers and growing media – Terminology*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in WI 00223137:–[3] and the following apply.

3.1

***Salmonella* spp.**

microorganisms which form typical colonies on solid selective media described and which display the morphological, physiological and biochemical characteristics described when the analysis is carried out in accordance with this document

3.2

segment sample

combination of all incremental samples taken from one segment for microbiological testing and is to be used as a laboratory sample.

Note1 to entry: There will be five segment samples taken from each sampled portion.

3.3

laboratory sample

representative part of the final sample prepared for testing

[1] Under preparation. Stage at time of publication: prEN 12579:202X.

[2] Under preparation. Stage at time of publication: prEN 13040-2:202X.

[3] Under preparation. Stage at time of publication: prCEN/TS XXXX:202X.

3.4

test sample

sample prepared from the laboratory sample (3.3) and from which test portions will be taken

3.5

test portion

quantity of material drawn from the test sample (or from the laboratory sample, if both are the same) and on which the tests or observations are actually carried out

4 Principle

[Note from the WG 5-secretariat: *Please note that the principle (Clause 4) is still under discussion within the working group.]*

4.1 General

The detection of *Salmonella* spp. requires four successive steps as specified in Annex A. The four steps are the pre-enrichment, the second enrichment, the isolation on a chromogenic agar, and the confirmation with a serological test (and if required, a selective media).

NOTE 1 *Salmonella* can be present in small numbers and are often accompanied by considerably larger numbers of other bacteria of the same family (*Enterobacteriaceae*) or of other families. Enrichment is used to allow the detection of low numbers of *Salmonella* or stressed *Salmonella*.

NOTE 2 Stressed microorganisms are defined here as those present in the environment that can be injured or that can have developed in harsh environments. Such organisms can be difficult to detect because they struggle to grow on selective media. However, under suitable conditions, they can repair the cellular damages and recover their normal properties.

NOTE 3 Validated alternative methods based on molecular biology may be used if giving the same results as those given in this document (or e.g. comparable to EN ISO 7218 [7]).

4.2 Pre-enrichment in liquid medium

Buffered peptone water (BPW) at room temperature is inoculated with the test portion (3.5), then incubated from 34 °C to 38 °C for 18 h ± 2 h.

4.3 For large quantities (e.g. 1 l or more), pre-warm BPW to 34 °C to 38 °C before mixing it with the test portion.Second enrichment in selective broth

A Rappaport-Vassiliadis Soja broth is inoculated with the BPW obtained in 4.2. This second enrichment is incubated at 42°C ± 2 °C for 24 h ± 3 h.

4.4 Plating out on selective solid media

From the second enrichment obtained in 4.2, the media (5.2) is inoculated and incubated at 36 °C ± 2 °C for 24 h ± 3 h (or according to the manufacturer's instructions if explicitly recommended).

4.5 Confirmation

Colonies of presumptive *Salmonella* are confirmed by means of appropriate serological test.

If the serological test gives a negative result, the inoculation of a selective agar (5.5) is required.

If the confirmation test gives a negative result, up to 4 other presumptive colonies will be tested (if possible and up to 5 colonies in total).

In case where identification of *Salmonella* spp. is unclear, other tests shall be carried out (e.g., PCR, biochemical strips.

5 Culture media and reagents

5.1 General

For current laboratory practice, standards comparable to EN ISO 7218 [7] and EN ISO 11133 [8] can be used.

Composition of culture media and reagents and their preparation are described in Annex B.

5.2 Rappaport-Vassiliadis Soya broth

The composition and volume of this selective broth are described in Annex B.3.

5.3 Isolation chromogenic agar

This isolation medium is chosen by the testing laboratory and shall highlight both the C8-esterase and the β-D-glucosidase enzymatic activities. For examples of isolation media, see Annex D, Table D.1.

[Note from the WG 5-secretariat: *The exact characteristics of the chromogenic medium need to be discussed in order to reach consensus.****]***

5.4 Non-selective agar

General purpose agar supporting the growth of a wide range of non-fastidious strains. See Annex B, clause B.4.

5.5 Confirmation selective agar

This confirmation medium is chosen by the testing laboratory and must highlight the production of hydrogen sulphide (H_2S) by the strains (see Annex B.5). For examples of confirmation media, see Annex D, Table D.2.

6 Equipment and consumables

Disposable equipment in an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment (e.g. EN ISO 7218 [7]) and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave). As specified in e.g. EN ISO 7218 [7].

6.2 Drying cabinet or oven, capable of operating between 25 °C and 50 °C .

6.3 Incubator(s), capable of operating in the range 34 °C to 38 °C or at 37 °C ± 1 °C.

6.4 Water bath, capable of operating at 47 °C to 50 °C, containing an antibacterial agent.

6.5 Cooling unit, adjustable at 5 °C ± 3 °C.

6.6 Freezer, capable of operating at -20 °C ± 5 °C.

6.7 Sterile loops of approximate diameter, 3 mm (10 µl volume).

6.8 pH-meter with a reading to the nearest 0,1 pH unit at 20 °C to 25 °C.

6.9 Sterile tubes, bottles, or flasks with caps of appropriate capacity.

6.10 Sterile filter with a pore size of 0,2 µm.

6.11 Sterile Petri dishes with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).

7 Sampling

Sampling shall have been conducted following EN 12579 [1].

8 Preparation of test sample

The test sample shall have been prepared following EN 13040-2 [2].

9 Procedure (see diagram in Annex A)

9.1 Test portion and initial suspension

For the preparation of the initial suspension, use as diluent the enrichment medium specified in Annex B.2 (buffered peptone water). Pre-warm the BPW to room temperature before use. A test portion (3.5) of 25 g or 25 ml is weighed and BPW (4.2) is added to yield a minimum tenfold dilution (mass or volume) (see EN 13040-2 [2]).

NOTE 1 It may be necessary to measure the pH of the initial suspension. If the samples are suspected to exhibit extreme pH values (pH lower than 4,5 and pH higher than 9, respectively), and correct the pH accordingly (e.g. use double buffered peptone water for the initial suspension for minor pH changes).

[Note from the WG 5-secretariat: *The precision of the size of the test sample (i.e. 25 g or 25 ml exact, or is there an error marge applicable?) needs to be confirmed.****]***

9.2 Pre-enrichment

Incubate the initial suspension (9.1) from 34 °C to 38 °C (6.3) for 18 h ± 2 h. It is allowed to keep the pre-enrichment culture at 5 °C (6.5) for 72 h maximum after the incubation.

9.3 Second enrichment

After the incubation, inoculate a Rappaport-Vassiliadis broth (5.2) with 0.1 ml of the initial suspension (9.2). Incubate RVS at 42°C ± 2 °C for 24 h ± 3 h. It is allowed to keep the selective enrichment at 5 °C (6.5) for 72 h maximum after the incubation.

9.4 Isolation

From the second enrichment (9.2), inoculate by means of a 10 µl loop (6.7) the surface of the chromogenic isolation agar (5.25.3) so that well-isolated colonies will be obtained. Allow the plates to equilibrate at room temperature if they were stored at a lower temperature. If necessary, dry the surface of the plates before use (e.g. EN ISO 11133 [8]). Incubate the selective plating-out medium from 34 °C to 38 °C (6.3) for 24 h ± 3 h (or according to the manufacturer's instructions if explicitly recommended). Typical colonies exhibiting the C8-esterase activity are smooth (or rarely rough), round and magenta. After incubation, check the chromogenic medium (5.2) for the presence of colonies which, from their characteristics, are considered to be suspect colonies.

9.5 Confirmation

9.5.1 General

The combination of enzymatic activity and serological test results indicate whether an isolate belongs to the genus *Salmonella* or not. For the characterization of *Salmonella* strains, full serotyping is needed. Guidance for serotyping is described in e.g. CEN ISO/TR 6579-3 [6]. For a clear distinction between positive and negative serological reactions, it is helpful to verify the reactions of the media of each biochemical test with well-characterized positive and negative control strains.

In case where identification of *Salmonella* spp. is unclear, other tests shall be carried out (e.g., PCR, biochemical strips.

9.5.2 Selection of colonies for confirmation

Mark suspect colonies on each plate (9.3). Select one suspect colony for confirmation. If this is negative, select up to four more suspect colonies (testing up to 5 colonies in total). If well-isolated colonies are available on the selective plating media (9.3), the serological and biological confirmation can be performed directly on a suspect colony. It is recommended to subculture this colony on a non-selective agar (5.44) to get a pure culture with enough material to work with.

9.5.3 Serological typing

9.5.3.1 General

The suspect colonies (9.5.2) are tested for auto-agglutination. Strains that are auto-agglutinable cannot be tested for the presence of *Salmonella* antigens and shall be confirmed by test for H_2S production (9.5.4). The suspect colonies are tested for the presence of *Salmonella* O-and H-antigens by slide agglutination using polyvalent antisera (Annex B.7). Use the antisera according to the manufacturer's instructions if different from the method described below to detect the presence of *Salmonella* O-and H-antigens. The following tests (9.5.3.2 to 9.5.3.5) are the minimum required for serological testing of *Salmonella* spp.

Note 1 Guidance on serological confirmation and on serotyping is given in e.g. CEN ISO/TR 6579-3 [6].

9.5.3.2 Elimination of auto-agglutinable strains

Place one drop of saline solution (Annex B.6) on a clean glass slide. Using a loop, disperse part of the colony to be tested in the saline to obtain a homogeneous and turbid suspension.

Rock the slide gently for 5 s to 60 s (depending on the manufacturer's instructions). Observe the suspension, preferably against a dark background. If the bacteria have formed granules in the suspension, this indicates auto-agglutination and serological confirmation will become complicated.

Additional information on the treatment of auto-agglutinating strains can be found in e.g. CEN ISO/TR 6579-3 [6].

9.5.3.3 Examination for O-antigens

Using one non-auto-agglutinating pure colony, proceed according to 9.5.3.2 using one drop of polyvalent anti-O sera (Annex B.7) in place of the saline solution.

If agglutination occurs, this is considered a positive reaction.

9.5.3.4 Examination for H-antigens

Using one non-auto-agglutinating pure colony, proceed according to 9.5.3.2 using one drop of polyvalent anti-H sera (Annex B.7) in place of the saline solution.

If agglutination occurs, this is considered a positive reaction.

9.5.3.5 Interpretation of serological reactions

Table 1 gives the interpretation of the confirmatory tests (9.5) carried out on the colonies used (9.5.2).

Table 1 – Interpretation of results of confirmatory tests

C8-esterase activity	Auto-agglutination	Serological reactions	Interpretation
Typical	No	O- and/or H-antigens positive	Detection
Typical	No	O- and/or H-antigens positive	Presumptive *Salmonella*: test for H_2S production (9.4.4)
Typical	Yes	Not tested because of auto-agglutination (see 9.4.3.2)	Presumptive *Salmonella*: test for H_2S production (9.4.4)
No typical reactions	-	-	Non detection

9.5.4 Selective medium for H_2S production

Some serotypes of *Salmonella* exhibit a very weak or do not exhibit an agglutination reaction (e.g. Arizonae, Diarizonae), therefore, in case of a negative serological reaction it is required to perform another confirmation test.

Streak the suspect colonies (9.5.2) onto the surface of a pre-dried selective agar (5.5) in a manner which will allow well-isolated colonies to develop. Incubate the plates from 34 °C to 38 °C for 24 h ± 3 h (6.3) (or according to the manufacturer's instructions if explicitly recommended).

The presence of typical colonies with a black centre on the selective confirmation media witnesses the detection of *Salmonella* spp. in the sample. The presence of colonies without blackening must be interpreted as the non-detection of *Salmonella* spp.

9.5.5 Biochemical testing (optional?)

[Note from the WG 5-secretariat: *Please note that the exact handling and types of biochemical tests is still under discussion within the working group.****]***

In the case of an unclear result regarding the identification of the strain, further biochemical tests can be carried out (e.g. urea, indole, ...).

9.5.6 Serotyping

If required, confirmed strains can be sent to a recognized *Salmonella* reference centre for definitive typing (serotyping, phage typing, molecular typing). If the strain is sent to a reference centre, it should be accompanied by all relevant information such as confirmation results and source from which the strain was isolated.

10 Expression of results

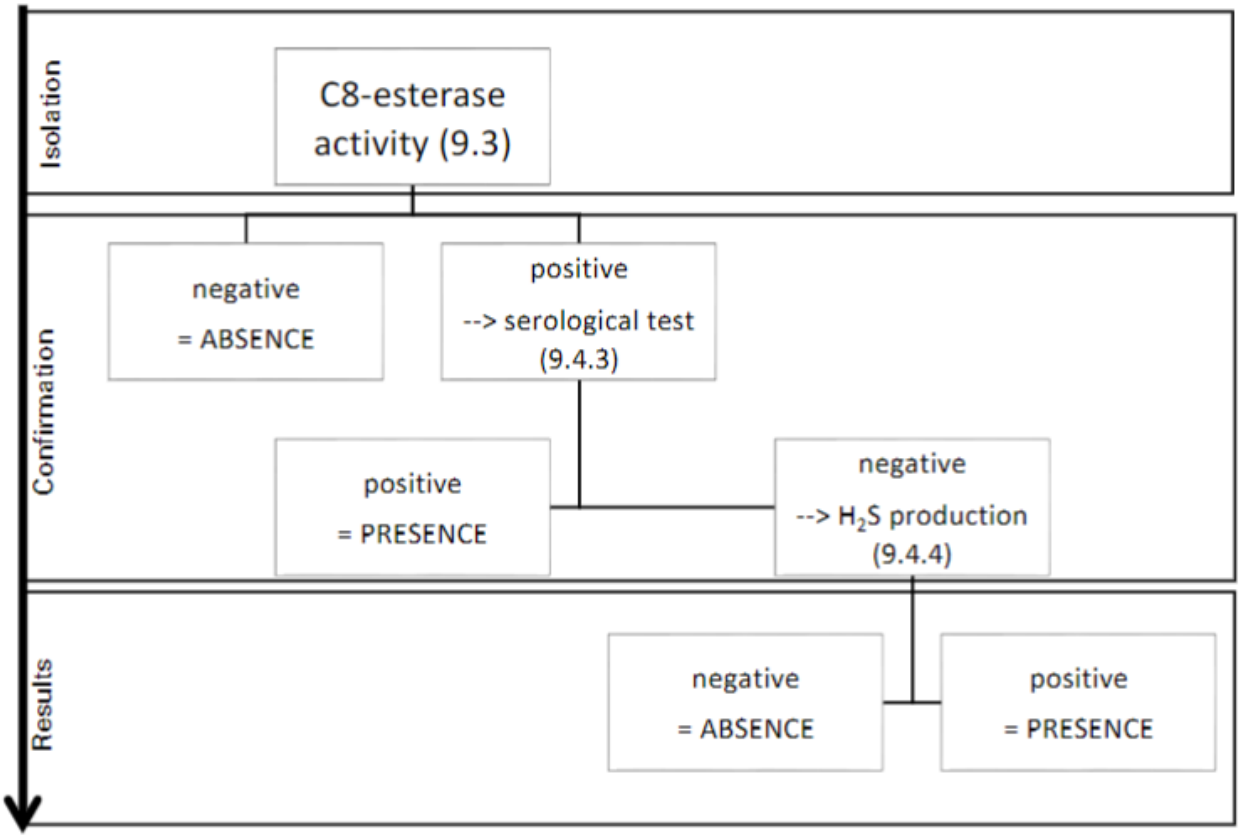

Figure 1 – Interpretation of the biological and serological results

In accordance with the interpretation of the results, indicate the presence or absence of confirmed *Salmonella* species in the test portion of 25 g or 25 ml of product.

11 Performance assays

Annex C to be later completed with the values derived from the further interlaboratory studies. These values may not be applicable to matrix types other than those given in Annex C. Furthermore, the performance characteristics as indicated in Annex C were determined with individual test portions of 25 g (or ml).

12 Test report

The test report shall specify the following:

—the sampling method used (EN 12579 [1]);

—the size of the test portion and/or the nature of the object examined;

—the test method used, with reference to this document;

—any deviation in the enrichment media or the incubation conditions used;

—all operating conditions not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the results;

—the results obtained, including a reference to the clause which explains how the results were calculated (see Clause 10);

—the date of the test.

Annex A
(normative)

Diagram of the procedures

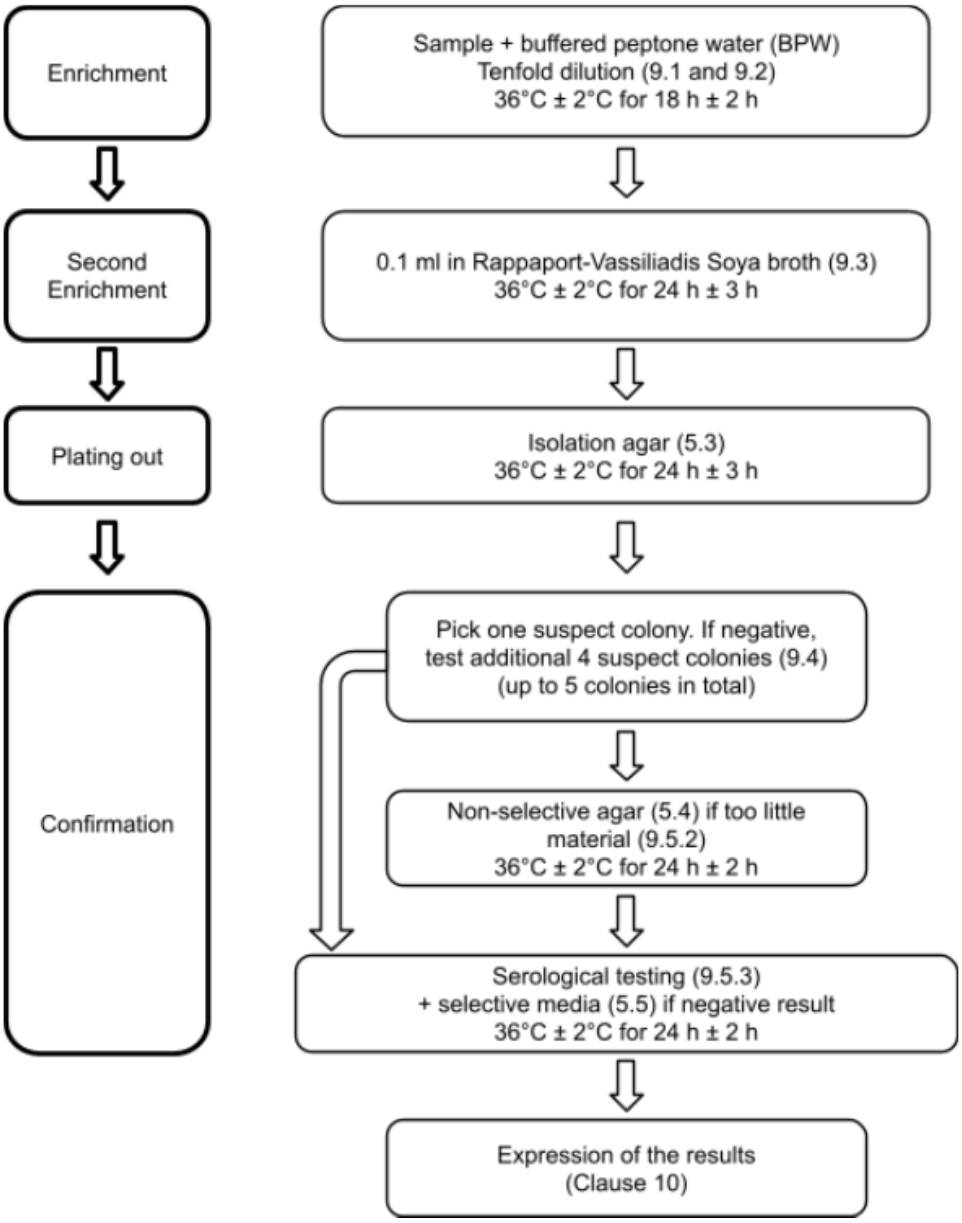

Figure A.1 – Diagram of procedure for the detection of *Salmonella* spp. in organic soil improvers and growing media

Annex B
(normative)

Culture media and reagents

B.1 General

The preparation and performance of culture media is a fundamental step to ensure the integrity of microbiological examination.

When ready-to-use media are used, the manufacturers of this available media should have a quality programme that ensures the quality of the media they supply, according to e.g. EN ISO 11133 [8]. Under this conditions, the user/laboratory not need to run additional testing on such media, but shall ensure the storage condition according to the ones recommended by the manufactures.

For diluents and media prepared by the user/laboratory directly from commercially available dehydrated formulations and/or from basic individual components, the performance of these diluents/media should be evaluated according to e.g. EN ISO 11133 [8].

B.2 Buffered peptone water (BPW)

B.2.1 Composition

Peptone [4]	10,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)[5]	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

NOTE In the case of double buffered BPW the double amount of phosphate salts has to be considered.

B.2.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH (6.8), if necessary, so that after sterilization, it is 7,0 ± 0,2 at 25 °C.

Dispense the medium into flasks (6.9) of suitable capacity to obtain the portions necessary for the test.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

Store the medium in closed containers (6.9) at 5 °C (6.5) for up to six months.

[4] For example, enzymatic digest of casein.

[5] If disodium hydrogen phosphate with a different water content is used, amend the mass of the ingredient accordingly. For example, in case of anhydrous disodium hydrogen phosphate (Na_2HPO_4), use 3,57 g.

B.3 Rappaport-Vassiliadis Soya broth

B.3.1 Composition

Papaic digest of soyabean meal	4,5 g
Sodium chloride	7,2 g
Potassium dihydrogen phosphate	1,26 g
Dipotassium phosphate	0,18 g
Magnesium chloride anhydrous	13,4 g
Malachite green	36 mg
Water	1000 ml

B.3.2 Preparation

Dissolve the components in the water by heating, if necessary.

Adjust the pH (6.8), if necessary, so that after sterilization, it is 5,2 ± 0,2 at 25 °C.

Dispense the medium into closed flasks (6.9) with a volume of 10 ml.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C.

Store the medium at 5 °C (6.5) for up to three months.

NOTE Commercially available RVS broth can be used.

B.4 Nutrient agar (example of non-selective medium)

B.4.1 Composition

Meat extract	3,0 g
Peptone	5,0 g
Sodium chloride (NaCl) (optional)	5,0 g
Agar	9 g to 18 g
Water	1 000 ml

B.4.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating, with frequent agitation.

Transfer the culture medium into tubes or flasks (6.9.) of appropriate capacity. Sterilize for 15 min in the autoclave (6.1) set at 121 °C. Adjust the pH (6.8), if necessary to 7,0 ± 0,2 at 25 °C.

B.4.3 Preparation of nutrient agar plates

Cool the medium to 47 °C to 50 °C in a water bath (6.4), mix, and pour into sterile Petri dishes (6.11). Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven (6.2) set between 25 °C and 50 °C until the surface of the agar is dry.

Store the poured plates protected from drying, at 5 °C (6.5.) for up to four weeks.

B.5 Triple sugar/iron agar (TSI agar; example for H2S production agar)

B.5.1 Composition

Meat extract	3,0 g
Yeast extract	3,0 g
Peptone	20,0 g
Sodium chloride (NaCl)	5,0 g
Lactose	10,0 g
Sucrose	10,0 g
Glucose	1,0 g
Iron(III) citrate	0,3 g
Sodium thiosulfate	0,3 g
Phenol red	0,024 g
Agar	9 g to 18 g[6]
Water	1 000 ml

B.5.2 Preparation

Dissolve the components or the dehydrated complete medium in the water by heating with frequent agitation.

Dispense the medium into tubes or bottles (6.9.) in quantities of 10 ml.

Sterilize for 15 min in the autoclave (6.1) set at 121 °C. Adjust the pH (6.8), if necessary to 7,4 ± 0,2 at 25 °C.

Allow to set in a sloping position to give a butt of depth 2,5 cm to about 5 cm. Store the poured tubes protected from drying, at 5 °C (6.5) for up to four weeks.

B.6 Saline solution

B.6.1 Composition

Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

B.6.2 Preparation

Dissolve the sodium chloride in the water.

Sterilize for 15 min in the autoclave (6.1.) set at 121 °C.

Adjust the pH (6.8), if necessary to 7,0 ± 0,2 at 25 °C.

[6] Depending on the gel strength of the agar.

Dispense the solution into flasks or tubes (6.9) of suitable capacity to obtain the portions necessary for the test.

Store the solution in closed flasks/tubes at 5 °C (6.5) for up to six months.

B.7 Antisera

Several types of agglutinating sera containing antibodies for one or several O-antigens are available commercially, i.e. antisera containing one or more "O" groups (called monovalent or polyvalent anti-O sera), and antisera containing antibodies for one or several H-factors (called monovalent or polyvalent anti-H sera). Commercially available *Salmonella* Latex tests can be used/are preferred in order to carry out the serological testing [SEE WITH TC].

B.8 Performance testing for the quality assurance of the culture media

The definition of selectivity and productivity is specified in e.g. EN ISO 11133 [8]. In general, follow the procedures for performance testing described in e.g. EN ISO 11133 [8]. For the other media, the inoculum levels for the target and the non-target organisms are specified in e.g. EN ISO 11133 [8].

Table B.8.1 – Performance testing for the quality assurance of the culture media

Medium	Function	Incubation	Control strains	WDCM numbers [a]	Criteria [b]
BPW	Productivity	18 h ± 2 h / 34 °C to 38 °C	*Salmonella* Typhimurium [c,d] *Salmonella* Enteritidis [c,d]	00031 00030	Turbidity (1–2)
Nutrient agar	Productivity	24 h ± 3 h / 34 °C to 38 °C	*Salmonella* Typhimurium [c,d] *Salmonella* Enteritidis [c,d]	00031 00030	Good growth
Confirmation media	Productivity	24 h ± 3 h / 34 °C to 38 °C	*Salmonella* Typhimurium [c,d] *Salmonella* Enteritidis [c,d]	00031 00030	Good growth, biochemical reactions

[a] Refer to the reference strain catalogue at www.wfcc.info for information on culture collection strain numbers and on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

[b] Growth is categorized as 0: no growth, 1: weak growth (partial inhibition), and 2: good growth (see e.g. EN ISO 11133 [8]).

[c] Some national restrictions and directions may require the use of a different serovar. Make reference to national requirements relating to the choice of Salmonella serovars.

[d] Strain free of choice; one of the strains has to be used as a minimum.

Annex C
(informative)

Method validation studies and performance characteristics

TO BE ADDED AT A LATER STAGE!

Annex D
(informative)

Examples of selective plating-out media

NOTE The lists of selective plating-out media are not exhaustive.

Table D.1 – Examples of isolation chromogenic medium for the expression of the C8-esterase activity

Media	Examples of manufacturers
ASAP® agar	Biomérieux
CHROMagar® Salmonella	Chromagar
ChromID® Salmonella	Biomérieux
CASE[a]	Neogen
Compass® Salmonella	Solabia
IRISa Salmonella®	Solabia
Salmonella ABC	LabM
Rapid'Salmo	Bio-rad
Salmonella Chromogenic agar	Oxoid
[a] CASE : Chromogenic Agar for Salmonella Esterase ; ABC : α β Chromogenic	

Table D.2 – Examples of confirmation selective medium highlighting the production of H_2S

Media	Examples of manufacturers
Bismuth Sulfite agar	Oxoid, Sigma-Aldrich, Merck millipore
Hektoen	Bio-rad
Kligler-Hajna	Bio-rad, Oxoid
Miller-Mallinson	Sigma-Aldrich, BD, VWR
Önöz	Merck
Shigella Salmonella	BD, Neogen, Oxoid, Solabia
TSI	BD, Oxoid, Bio-rad
XLD	Solabia

Preparation and storage of the media must be carried out in accordance with the manufacturer's instructions.

NOTE Commercially available media can be used if the biochemical reactions fulfil the requirements stated in Table D.1 and Table D.2.

Bibliography

[1] EN 12579, Soil improvers and growing media - Sampling

[2] EN 13040-2, Soil improvers and growing media – Part 2: Sample preparation for microbiological examination

[3] WI 00223137: –, Soil improvers and growing media – Terminology

[4] EN ISO 6579-1, Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. (ISO 6579-1)

[5] EN ISO 6579-1:2017/A1:2020, Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 1: Detection of *Salmonella* spp. AMENDMENT 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC (ISO 6579-1:2017/Amd 1:2020)

[6] CEN ISO/TR 6579-3, Microbiology of the food chain – Horizontal method for the detection, enumeration and serotyping of *Salmonella* – Part 3: Guidelines for serotyping of *Salmonella* spp. (ISO/TR 6579-3)

[7] EN ISO 7218, Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

[8] EN ISO 11133, Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133)

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