CEN/TC 223

Date: 2022-05 - 04

2nd working draft WI 00223114 prEN XXXXX:XXXX

Document **CEN/TC 223/WG 5/N 0154a**

Secretariat: NEN

Soil improvers and growing media —Enumeration of *Escherichia coli*

Einführendes Element — Haupt-Element — Ergänzendes Element

Élément introductif — Élément central — Élément complémentaire

ICS:

CCMC will prepare and attach the official title page.

Contents

Page

European foreword 3
Introduction 4
1 Scope 5
2 Normative references 5
3 Terms and definitions 5
4 Principle 7
5 Diluents, culture media and reagents 7
6 Equipment and consumables 8
7 Sampling 8
8 Preparation of test sample 9
9 Procedure (see Figure A.1 in Annex A) 9
10 Expression of results 11
11 Method validation 12
12 Test report 12
Annex A (normative) 13
Annex B (normative) Composition and preparation of culture media and reagents 14
B.1 Basic phosphate buffer 14
B.2 double-buffered phosphate buffer 14
B.4 Tryptone-bile-X-glucuronic medium (TBX) 15
B.5 MacConkey Agar No. 3 (optional) 15
B.6 Tryptone/tryptophan medium (optional) 16
B.7 Kovacs reagent 17
Annex C (informative) Method validation studies and performance characteristics 18
Bibliography 19

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 standardization request given to CEN by the European Commission and the European Free Trade Association.

Introduction

This document describes a method for the detection and enumeration of *Escherichia coli* in soil improvers and growing media.

This methodology has been developed to detect and enumerate *Escherichia coli* in organic soil improvers and growing media in order to be able to control certain hygienic requirements. The method is based on ISO 16649-2 [4].

Escherichia coli (*E. coli*) is a Gram negative bacterium of faecal origin and consists of a large number of different strains. 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.

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 [9].

1 Scope

This document specifies a method for the enumeration of *Escherichia coli* (*E. coli*) in soil improvers, soil improver blends, growing media and growing media blends. This document applies to material in solid form (including pre-shaped growing media) and liquid form.

This document specifies a colony-count technique at 44 °C on a solid medium containing a chromogenic ingredient for the detection of the enzyme ß-glucuronidase.

Strains of *Escherichia coli* which do not grow at 44 °C and, in particular, those that are ß-glucuronidase negative, such as *Escherichia coli* O157, will not be detected. Detected microorganisms are considered as presumptive β-glucuronidase positive *Escherichia coli*, since some Enterobacteriaceae, in particular *Shigella* and *Salmonella*, can also show β-glucuronidase activity at 44 °C.

In the case the given limit value (e.g. [1]) is reached, a confirmation step (indole reaction) shall be performed.

NOTE: This method has been validated in an interlaboratory study for the following soil improver and growing media materials:

— To be filled in after CEN/TC 223 decides on the materials to be used.

— Etc.

Validations have been performed with specific materials that were present on the market during the validation study. Materials/formulation can change over time regarding their composition.

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

3.1

segment sample

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

[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.

Note 1 to entry: There will be five segment samples taken from each sampled portion.

3.2

laboratory sample

representative part of the final sample prepared for testing

3.3

test sample

sample prepared from the laboratory sample (3.2) and from which test portions will be taken

3.4

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

3.5

initial suspension

primary dilution obtained after a weighed or measured quantity of the product under examination (or of a test sample prepared from the product) has been mixed with, normally, a nine-fold quantity of diluent

Note 1 to entry: A closer ratio between the diluent and the quantity of product is often not recommended because of possible inhibiting influences of the matrix.

3.6

further dilutions

suspensions or solutions obtained by mixing a measured volume of the initial suspension (3.5) with an x-fold volume of diluent and by repeating this operation with further dilutions until a dilution series, suitable for the inoculation of culture media, is obtained

Note 1 to entry: Ten-fold dilutions are normally used to produce a decimal dilution series, but other ratios may be required for specific purposes.

3.7

ß-glucuronidase positive presumptive *Escherichia coli*

bacteria which at 44 °C form typical blue colony on tryptone-bile-X-glucuronide medium (TBX) under the conditions specified in this method

3.8

confirmed ß-glucuronidase positive *Escherichia coli*

ß-glucuronidase positive presumptive *Escherichia coli* showing a positive indole reaction in tryptophan broth under the conditions specified in this method

3.9

enumeration of ß-glucuronidase positive *Escherichia coli*

determination of the number of colony-forming units (CFU) of ß-glucuronidase positive *Escherichia coli*, per milliliter or per gram of sample, when test, confirmation and calculations are carried out in accordance with this method

4 Principle

Duplicate plates of tryptone-bile-glucuronic medium (TBX) are inoculated and incubated for 18 h to 24 h at 44 °C ± 1 °C. The plates are thereafter examined to enumerate presumptive *E. coli* colonies.

5 Diluents, culture media and reagents

5.1 General

For standard laboratory practice, standards comparable to e.g. EN ISO 7218 [7] and EN ISO 11133 [3] can be used.

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

For uniformity of results, in the preparation of media, either use a dehydrated complete medium, or a commercial ready-to-use agar, or use constituents of uniform quality and chemicals of recognized analytical grade.

5.2 Diluents

5.2.1 General

Some materials can significantly change the pH value of the initial suspension, which can negatively affect the viability of the microorganisms under investigation. In general, a basic phosphate buffer is sufficient to prepare the initial suspension. When testing bog peat, *Sphagnum*, alkaline composts or blended products with a high content of minerals, the pH of the initial suspension shall be checked. In these cases, the use of a double-buffered phosphate buffer is recommended.

NOTE: In the case materials containing mineral components are brought into solution, pH should be corrected to a pH between 6 and 8.

[Comment from the WG 5-secretary: *Please note that the consequence of the pH should be tested in trials. And ISO 6887 (all parts except for Part 4) should be checked on this matter****].***

If the substrate is simultaneously tested for the presence of *Salmonella* (WI 00223121:–[4]), the initial suspension for enrichment can be used with buffered or double-buffered peptone water (with 10 g peptone). In this case, rapid processing of the initial suspension is necessary.

5.2.2 Basic phosphate buffer

See Annex B.1.

5.2.3 Double-buffered phosphate buffer

See Annex B.2.

5.1.3 Liquids for reconstitution

See Annex B.3.

[4] Under preparation. Stage at time of publication: prENXXXX:202X.

5.3 Culture media

5.3.1 Tryptone-bile-X-glucuronic medium (TBX)

See Annex B.4.

5.3.2 MacConkey Agar No. 3 (optional)

See Annex B.5.

5.3.3 Medium and reagent for indole reaction (optional)

5.3.3.1 Tryptone/tryptophan medium

See Annex B.6.

5.3.3.2 Kovacs reagent

See Annex B.7.

6 Equipment and consumables

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

Usual microbiological laboratory equipment (described in standards comparable to e.g. EN ISO 7218 [7]) can be used 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 Incubator(s), capable of maintaining a temperature of 44 °C ± 1 °C, or optionally capable of maintaining a temperature of 37 °C ± 1 °C and/or 35 °C ± 1 °C and/or 44 °C to 47 °C.

6.3 Blending equipment, as described in EN 13040-2 [9] shall be used.

6.4 Scales, of the required range and accuracy for the different products to be weighed.

6.5 Water bath, capable of maintaining a temperature between 44 °C to 47 °C.

6.6 Cooling unit, adjustable to 5 °C ± 3 °C.

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

6.8 Sterile loops, with an approximate diameter of 3 mm (**10 µl volume).**

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

6.10 Pipettes or pipettor and sterile tips of nominal capacities of 10 ml and 1 ml.

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

7 Sampling

Sampling shall have been conducted following EN 12579 [2].

8 Preparation of test sample

The test sample shall have been prepared following EN 13040-2 [9].

9 Procedure (see Figure A.1 in Annex A)

9.1 Preparation of the initial suspension and decimal dilutions

Preparation of the initial suspension and further decimal dilutions shall have been performed according to EN 13040-2 [9].

If the initial suspension is also used for detection of *Salmonella*, the amount of sample has to be adapted, for example weigh 26 g into a sterile container and add 234 ml buffered peptone water or double-buffered peptone water.

NOTE: In the case materials containing mineral components are brought into solution, pH should be corrected to a pH between 6 and 8.

[Comment from the WG 5-secretary: *Please note that the consequence of the pH should be tested in trials. And ISO 6887 (all parts except for Part 4) should be checked on this matter****].***

9.2 Inoculation and incubation

9.2.1 General

The time elapsing between the start of the preparation of the initial suspension and the moment when the plates are inoculated shall not exceed 45 min.

To check sterility, prepare a control pour plate.

Cultivation of an aliquot of the initial suspension is only possible if no toxic or inhibiting substances are expected in the sample. For blended products with high amounts of minerals, it is recommended to use aliquots from the first dilution (10^{-2}) and higher. Liquid materials can be used undiluted for cultivation if they do not contain any toxic or inhibiting substances.

9.2.2 Procedure

Using a sterile pipette or a micropipette (6.11), transfer 1 ml of the test sample (if liquid), or 1 ml of the initial dilution (10^{-1}) in the case of other products to a sterile Petri dish (6.12).

Inoculate two plates per dilution.

Repeat the procedure with further decimal dilutions, if necessary, using a new sterile pipette for each dilution.

NOTE: Where greater dilutions are made, the number of inoculated plates for enumeration tests should be increased to ensure a minimum 0,1 g of the test portion is distributed between all plates when low counts are expected. If the colony counts are expected to be close to the given limit value (e.g. [1]), it is recommended to increase the accuracy of the method by adding10 ml distributed into two Petri dishes with a diameter of 140 mm, or at least three Petri dishes with a diameter of 90 mm.

[Comment from the WG 5-secretary: *Please note that this note above differs from the same note given in the 2nd working draft for WI 00223119 'Enterococcaceae'. Is that okay, or should these notes be aligned between these drafts?****].***

[Comment from the WG 5-secretary: *Please note that it needs to be tested if 10 ml can be distributed in 3 90 mm Petri dishes.****].***

Pour into each Petri dish approximately 15 ml (for 90 mm Petri dishes), or 45 ml (for 140 mm Petri dishes) of the TBX medium (5.3.1), cooled down to 44 °C to 47 °C in the water bath (6.6) or the incubator (6.2).

Carefully mix the inoculum with the medium and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface.

The time which elapses between the inoculation of the empty Petri dish and pouring of the medium shall not exceed 15 min.

Incubate the inoculated dishes upside down in an incubator (6.2) set at 44 °C ± 1 °C for 21 h ± 3 h.

9.3 Enumeration of colonies

After incubation under the conditions specified above, all plates showing less than 150 typical ß-glucuronidase positive presumptive *Escherichia coli* and less than 300 total (typical and non-typical) colonies are used for the enumeration.

9.4 Confirmation

When embedded in agar, typical presumptive ß-glucuronidase positive *Escherichia coli* appear as strong dark to light blue colonies with a diameter greater than 1 mm. On the surface of the TBX-agar, typical colonies appear blue in centre with a white edge.

Confirmation is only required if the calculated result of presumptive ß-glucuronidase positive *Escherichia coli* exceeds the given limit value (e.g. [1]). Typical colonies picked for confirmation may be restreaked on TBX medium and inoculated upside down in an incubator set at 44 °C ± 1 °C for 21 h ± 3 h to create back-up material.

NOTE: Any other method capable of unambiguously identifying *E. coli* than the one described below may be used on typical colonies (e.g. commercial available biochemical test kits, serological confirmation, polymerase chain reaction (PCR)-analysis or via Matrix-Assisted Laser Desorption Ionization - Time-of-Flight Mass Spectrometry (MALDI-TOF)).

9.4.1 Indole reaction

The indole test can be used to differentiate *Salmonella* and *Enterobacter* (indole negative) from *Escherichia coli* (indole positive).

Pick at least 5 typical colonies from each countable plate with a sterile loop (6.9) and inoculate 5 ml of tryptone/tryptophan medium (5.2.3.1) with each suspected colony. In the case < 5 typical colonies are present, all colonies shall be picked.

[Comment from the WG 5-secretary: *A comment proposing to pick exactly 5 typical colonies was entered by the Netherlands. Piet Derikx and Henriette Mietke agreed to contact each other to discuss this matter and will propose their outcome to WG 5 (action 2022-23)****].***

Incubate at 37 °C ± 1 °C (6.2) for 24 h ± 3 h. After incubation, add 1 ml of the Kovacs reagent (5.2.3.2).

The formation of a red ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

9.4.2 Subculture

If it is not possible to isolate a single colony for the indole test due to a high background flora, a pure culture shall be established by subcultivation. With a sterile loop prepare streak plates from up to

5 typical colonies from each countable plate on poured TBX agar (5.2.1) or MacConkey agar No. 3 (5.2.2) yielding single colonies after incubation.

NOTE: Any other medium giving a clear distinction between *Escherichia coli* and other bacteria is applicable.

Incubate TBX agar (5.2.1) at 44 °C ± 1 °C for 21 h ± 3 h or MacConkey agar No. 3 at 35 °C ± 1 °C for 21 h ± 3 h. Typical colonies on TBX agar show a blue centre with a white edge. While non-lactose fermenters form colourless colonies on MacConkey agar No. 3, coliform bacteria appear as intense violet-red colonies and colonies typical for *E. coli* additionally shows bile precipitation whilst. Typical colonies can be subjected to a subsequent tryptophan test.

10 Expression of results

Results shall be checked according to general instructions stated in ISO 7218 [7].

The number of colony forming units (CFU) per gram or per ml test portion is calculated using Formula 1 [7].

$$N = \frac{\sum C}{V\left(n_1 + 0{,}1 n_2\right) d} \tag{1}$$

where

ΣC is the sum of the colonies counted on all considered Petri dishes from one or two successive dilutions;

V is the volume of inoculum applied to each Petri dish, in ml;

n_1 is the number of dishes retained at the first dilution;

n_2 is the number of dishes retained at the second dilution ($n_2 = 0$ if only one dilution is considered);

d is the dilution factor corresponding to the more concentrated dilution retained (d = 1 for undiluted liquid product).

When confirmation is required, a given number A (generally 5 per plate) of presumptive colonies are confirmed from each of the dishes retained for counting.

After confirmation calculate, for each of the dishes, the number of colonies (a) complying with identification criteria using Formula (2).

$$a = \frac{b}{A} \times C \tag{2}$$

where

A is the number of presumptive colonies selected for confirmation from each Petri dish;

b is the number of colonies confirmed from A;

C is the total number of colonies in the Petri dish.

And replace ΣC by Σa in Formula (1) to account for the ratio of verified colonies.

NOTE If Σa is < 10, the final result is expressed as an estimated value.

11 Method validation

The method is validated in an interlaboratory study for the following products (*Escherichia coli* was investigated in both native and spiked test material):

Table 1 — Product groups and matrices for which the methods described in this method are applicable and tested in a validation trial

Product group	Matrix
Organic soil improvers	to be determined at EN phase
Growing media	to be determined at EN phase

International ring trials to be addressed at the EN phase.

12 Test report

The test report shall specify:

- any deviations from sampling method EN 12579 [2] shall be stated;
- the size of the test portion and/or the nature of the object examined;
- the test method used, with reference to this document (EN XXXX:202X);
- any deviation in the enrichment media or the incubation conditions used;
- any unusual features observed;
- 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 (see 10) calculated
- the date of the test.

Annex A
(normative)

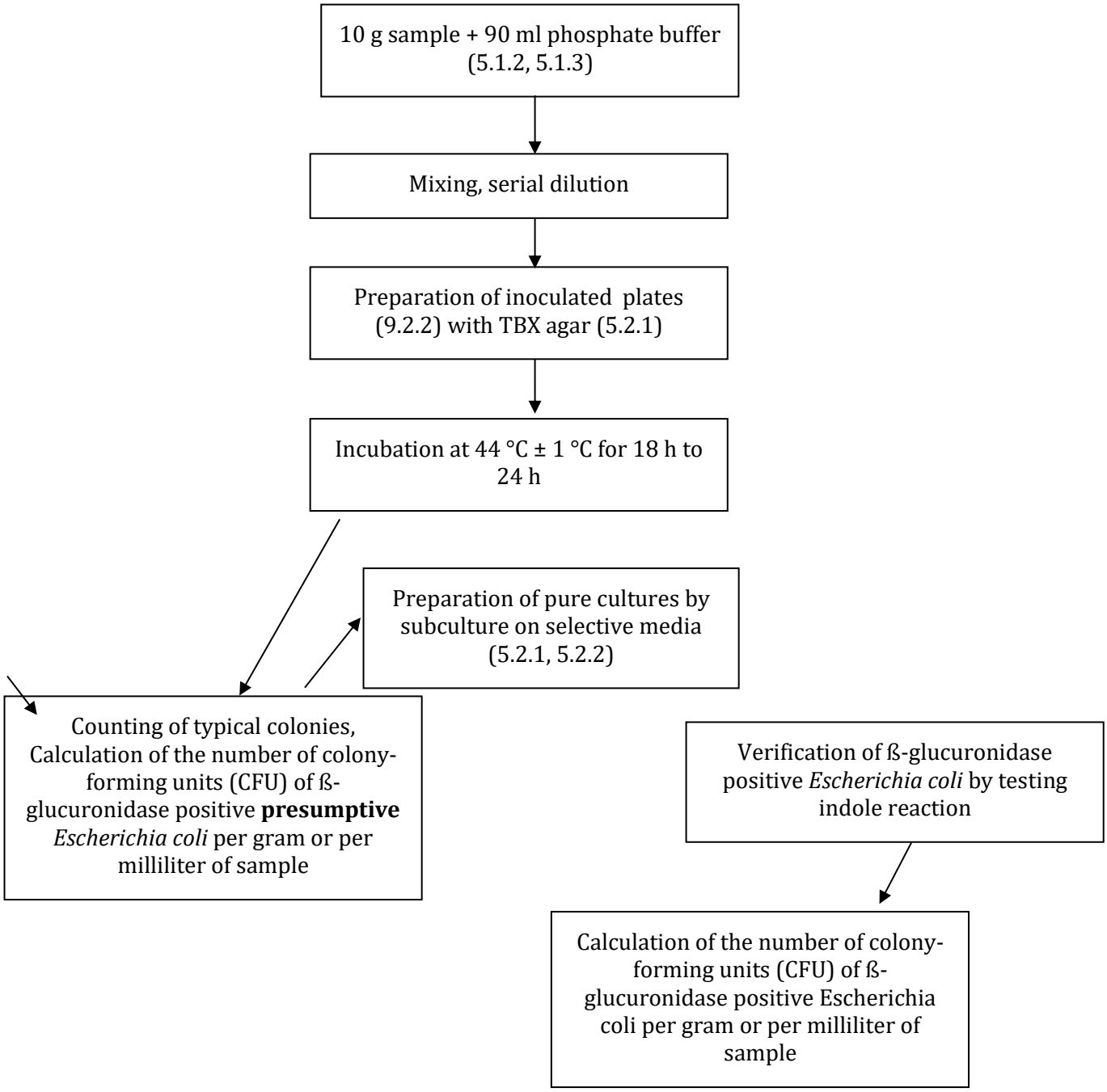

Figure A.1 — Flow diagram of the method for the determination of *Escherichia coli* in organic soil improvers and growing media

Annex B
(normative)

Composition and preparation of culture media and reagents

If media or reagents are prepared from dehydrated complete media/reagents or if ready-to-use media/reagents are used, follow the manufacturer's instructions regarding preparation, storage conditions, expiry date and use.

The shelf lives of the media indicated in this annex have been shown in some studies. The user should verify this under their own storage conditions (as described in standards comparable to e.g. EN ISO 11133 [3]).

B.1 Basic phosphate buffer

B.1.1 Composition

Peptone[a]	1,0 g
Sodium chloride	5,0 g
Disodium hydrogen phosphate dodecahydrate ‡ ($Na_2HPO_4 \cdot 12H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4) ‡	1,5 g
Water	1 000 ml
[a] For example, enzymatic digest of casein.	

NOTE When using buffered peptone water (BPW), the peptone content increases to 10 g/l.

Commercially available, ready-to-use BPW medium is also suitable.

B.1.2 Preparation

Dissolve the components in the water in flasks, bottles or test tubes (6.10), by heating if necessary.

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

B.2 double-buffered phosphate buffer

This buffer contains twice the amount of the two buffer components (marked ‡)

NOTE When using double buffered peptone water, the peptone content increases to 10 g/l.

Commercially available, ready-to-use medium is suitable.

B.3 Solutions for reconstitution

B.3.1 Tap water

Pour tap water into bottles or flasks (6.10) and sterilize it in the autoclave (6.1) set at 121 °C ± 3 °C for 15 min.

B.3.2 Physiological saline

B.3.2.1 Composition

Sodiumchloride NaCl	9,5 g
Water	1000 ml

Commercially available, ready-to-use physiological saline is also suitable.

B.3.2.2 Preparation

Dissolve the salt in the water in flasks or bottles (6.10) and sterilize it in the autoclave (6.1) set at 121 °C ± 3 °C for 15 min.

B.4 Tryptone-bile-X-glucuronic medium (TBX)

B.4.1 Composition

Enzymatic digest of casein	20,0 g
Bile salts No. 3	1,5 g
5-Bromo-4-chloro-3-indolyl ß-D-glucuronic acid (BCIG), Cyclo hexylammonium salt monohydrate (CAS N° 114162-64-0)	144 µmol[a]
Agar	9 g to 18 g[b]
Water	1 000 ml

[a] e.g. 0,075 g of cyclohexylammonium salt.

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

B.4.2 Preparation

Dissolve the BCIG in the dimethyl sulfoxide or in the diluent recommended by the manufacturer. Dissolve all components in the water and heat to boiling.

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

Sterilize the medium in the autoclave (6.1) set at 121 °C ± 3 °C for 15 min. Immediately cool the medium in the water bath (6.6) at 44 °C to 47 °C up to the use in pour plate method. For the production of plates for the confirmation reaction cool the medium to 44 °C to 47 °C in a water bath (6.6), mix and pour into sterile Petri dishes (6.12). Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) until the surface of the agar is dry.

Store the poured plates, protected for drying, at 5 °C ± 3 °C (6.7) for up to 4 weeks.

B.5 MacConkey Agar No. 3 (optional)

A selective medium giving excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram positive enterococci.

B.5.1 Composition

Peptone	20,0 g
Lactose	10,0 g
Bile salts	1,5 g
Sodium chloride (NaCl)	5,0 g
Neutral red	0,03 g
Crystal violet	0,001 g
Agar	9 g to 18 g[a]
Water	1000 ml

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

B.5.2 Preparation

Dissolve all components in the water and heat to boiling.

Adjust the pH (6.8), if necessary, so that after sterilization, it is 7,1 ± 0,2.

Sterilize the medium in the autoclave (6.1) set at 121 °C ± 3 °C for 15 min. Immediately cool the medium in the water bath (6.6) at 44 °C to 47 °C, mix and pour into sterile Petri dishes (6.12). Allow to solidify.

Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) until the surface of the agar is dry.

Store the poured plates, protected for drying, at 5 °C ± 3 °C (6.7) for up to 4 weeks.

B.6 Tryptone/tryptophan medium (optional)

B.6.1 Composition

Tryptone	10,0 g
Sodium chloride (NaCl)	5,0 g
DL-Tryptophan	1,0 g
Water	1 000 ml

B.6.2 Preparation

Dissolve the components in water and heat to boiling whilst stirring continuously to dissolve all ingredients completely. Adjust the pH, if necessary, so that after sterilization it is 7,5 ± 0,2 at 25 °C.

Dispense 5 ml of the medium into each of several tubes (6.10).

Sterilize for 15 min in the autoclave (6.1) set at 121 °C ± 3 °C. Store the poured tubes at 5 °C ± 3 °C (6.7) for up to 3 months.

B.7 Kovacs reagent

B.7.1 Composition

4-Dimethylaminobenzaldehyde	5,0 g
Hydrochloric acid, mass concentration ρ = 1,18 g/ml to 1,19 g/ml	25 ml
2-Methylbutan-2-ol	75 ml

B.7.2 Preparation

Mix the components. Store the complete reagent, in closed flasks in the dark, at 5 °C ± 3 °C (6.7) for up to 6 months.

Annex C
(informative)

Method validation studies and performance characteristics

To be filled in after validation studies.

Bibliography

[1] Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003

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

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

[4] ISO 16649-2:2001, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*

[5] EN ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

[6] EN ISO 6887-1:2017, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions (ISO 6887-1:2017)*

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

[8] EN ISO 18593, *Microbiology of the food chain — Horizontal methods for surface sampling*

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