BS EN 17711:2024

BSI Standards Publication

Plant biostimulants — Detection of *Vibrio* spp.

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National foreword

This British Standard is the UK implementation of EN 17711:2024. It supersedes PD CEN/TS 17711:2022, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee W/-, Consumer Products and Services Sector Policy and Strategy Committee.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Plant biostimulants - Detection of *Vibrio* spp.

Biostimulants des végétaux - Détection de *Vibrio* spp.

Pflanzen-Biostimulanzien - Nachweis von *Vibrio* spp.

This European Standard was approved by CEN on 26 August 2024.

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European foreword

This document (EN 17711:2024) has been prepared by Technical Committee CEN/TC 455 "Plant biostimulants", the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 17711:2022.

EN 17711:2024 includes the following significant technical changes with respect to CEN/TS 17711:2022:

— the European foreword and Introduction have been updated;

— normative references have been updated;

— Table 1 has been updated;

— in 5.5, reagents list for biochemical tests has been revised;

— Clause 7 and Clause 8 have been updated;

— Table 2 has been revised;

— 9.5.4.1 and 9.5.4.6 have been revised;

— Table 4 has been revised;

— Clause 10 has been revised;

— Annex ZA has been added;

— the Bibliography has been updated.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

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Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

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1 Scope

This document specifies a horizontal method for the detection of enteropathogenic *Vibrio* species (spp.), which causes human illness in or via the intestinal tract. The species detectable by the methods specified include *Vibrio parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus*.

It is applicable to the microbial plant biostimulants.

NOTE 1 The World Health Organization (WHO) has identified that *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are the major contaminants of *Vibrio* spp. [2].

NOTE 2 For confirmation, it is possible to use PCR (Polymerase Chain Reaction) tests; in this case a validation is carried out by the laboratory for the procedure and data generated.

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Plant Biostimulants, and where the following category Plant Biostimulants is the highest % in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest % in the blend, the European Standard for the highest % of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

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 17702-1:2024, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

EN 17724:2024, *Plant biostimulants — Terminology*

EN ISO 7218:2024, *Microbiology of the food chain — General requirements and guidance for microbiological examinations (ISO 7218:2024)*

EN ISO 11133:2014[2], *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media (ISO 11133:2014)*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

[1] An example of such a blend is a product with 2 claimed functions consisting of a non-microbial plant biostimulant and an organic fertilizer composed of 1kg/kg of plant biostimulant from seaweed.
[2] As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020

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3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 17724:2024 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
potentially enteropathogenic *Vibrio* spp.
microorganism which forms typical colonies on solid selective media and which possesses the described biochemical or molecular characteristics when the test is performed in accordance with this document

Note 1 to entry: This document describes specific procedures for *V. parahaemolyticus, V. cholerae* and *V. vulnificus.*

3.2
detection of potentially enteropathogenic *Vibrio* spp.
determination of the presence or absence of potentially enteropathogenic *Vibrio* spp. (3.1) *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* in a determined quantity of product, when the test is performed in accordance with this document

4 Principle

4.1 General

The detection of potentially enteropathogenic *Vibrio* spp. *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* requires four successive phases, as shown in the procedure diagram in Annex A. Recovery of certain *Vibrio* spp. is improved by the use of different incubation temperatures depending upon the target species or state of the matrix. In liquid products, recovery of *V. parahaemolyticus* and *V. cholerae* is enhanced by enrichment at 41,5 °C and recovery of *V. vulnificus is* enhanced by enrichment at 37 °C. Whereas in solid products, for *V. vulnificus, V. parahaemolyticus* and *V. cholerae* recovery is enhanced by enrichment at 37 °C.

If detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* is required, all specified incubation temperatures shall be used. If detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* together is not required, the specific procedure(s) is selected according to the species being sought. Such a selection shall be clearly specified in the test report.

V. parahaemolyticus, *V. cholerae* and *V. vulnificus* can be present in small numbers and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or to other families.

4.2 Primary enrichment in a liquid selective medium

Inoculation of the test portion in the primary enrichment medium alkaline saline peptone water (ASPW) (5.1) at ambient temperature shall be followed by incubation at 41,5 °C for 6 h and/or 37 °C for 6 h. The incubation conditions are determined by the target species and product state.

For detection of all target species in solid products, primary enrichment shall be at 37 °C.

For detection of *V. vulnificus* in all products, primary enrichment shall be at 37 °C.

For detection of *V. parahaemolyticus* and/or *V. cholerae* only, in liquid products, primary enrichment shall be at 41,5 °C.

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4.3 Secondary enrichment in a liquid selective medium

The second enrichment medium (ASPW) shall be inoculated with the cultures obtained in 4.2. Incubation of inoculated enrichment medium shall be at 41,5 °C for 18 h and/or 37 °C for 18 h.

For detection of *V. vulnificus* in all products, secondary enrichment shall be at 37 °C.

For detection of V. *parahaemolyticus* and/or *V. cholerae* only, in all products, secondary enrichment shall be at 41,5 °C.

4.4 Isolation and identification

From the cultures obtained in 4.2 and in 4.3, inoculation of two solid selective media shall be done:

— thiosulfate citrate bile and sucrose agar (TCBS) medium (5.2.1);

— another appropriate solid selective medium (left to the choice of the laboratory), such as chromogenic agar, complementary to the TCBS medium (5.2.2).

Incubation of the TCBS medium shall be at 37 °C, then examination after 24 h. Incubation of the second selective medium shall follow the manufacturer's recommendations.

4.5 Confirmation

Presumptive colonies of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* isolated in 4.4 shall be subcultured and confirmed by means of an appropriate biochemical test. The PCR test is also possible to use for confirmation; the PCR methods are suggested in Annexes C and D, but the laboratory must validate the procedure and the data generated.

5 Culture media and reagents

For general laboratory practice, EN ISO 7218:2024 shall be used as reference.

For clarity of the text, details of the composition of culture media and reagents and their preparation are described in Annex B.

For performance testing of culture media, EN ISO 11133:2014 shall be used as reference.

5.1 Enrichment medium: alkaline saline peptone water (ASPW)

As specified in B.3.

5.2 Solid selective isolation media

5.2.1 First medium: thiosulphate citrate bile and sucrose agar (TCBS) medium

As specified in B.4. According to Table 1 for performance testing data.

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Table 1 — Performance testing of thiosulphate citrate bile and sucrose agar (TCBS) medium

Function	Incubation	Control strains	WDCM [a]	Method of control	Criteria [b]	Characteristic reactions
Productivity	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio parahaemolyticus*	00185 [c]	Qualitative	Good growth (2)	Green colonies (sucrose negative)
	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio furnissii*	00186 [c]	Qualitative	Good growth (2)	Yellow colonies (sucrose positive)
Selectivity	37 °C ± 1 °C for 24 h ± 3 h	*Escherichia coli* [d e]	00012, 00013 or 00090	Qualitative	Total inhibition (0)	—

[a] World Data Centre for Microorganisms (WDCM) strain catalogue available at http://refs.wdcm.org

[b] Growth is categorized as 0: no growth, 1: weak growth (partial inhibition), and 2: good growth (see EN ISO 11133:2014).

[c] Strain to be used as a minimum (see EN ISO 11133:2014).

[d] Strain free of choice; one of the strains shall be used as a minimum (see EN ISO 11133:2014).

[e] Some national restrictions and directions can require the use of a different *E. coli* serovar. National requirements relating to the choice of *E. coli* serovars shall be followed.

5.2.2 Second medium

The selection of the second medium is left to the choice of the test laboratory. The medium shall be prepared strictly according to the manufacturer's instructions.

5.3 Saline nutrient agar (SNA)

As specified in B.5.

5.4 Reagent for detection of oxidase

As specified in B.6.

5.5 Reagent for biochemical tests

5.5.1 L-lysine decarboxylase saline medium (LDC)

As specified in B.7.

5.5.2 Arginine dihydrolase saline medium (ADH)

As specified in B.8.

5.5.3 Reagent for detection of β-galactosidase

As specified in B.9.

5.5.4 Saline medium for detection of indole

As specified in B.10.

5.5.5 Saline peptone water

As specified in B.11.

5.5.6 Sodium chloride solution

As specified in B.12.

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