

STN	Rastlinné biostimulátory Detekcia <i>Vibrio</i> spp.	STN EN 17711 46 5612
------------	---	--

Plant biostimulants - Detection of *Vibrio* spp.

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 02/25

Obsahuje: EN 17711:2024

Oznámením tejto normy sa ruší
STN P CEN/TS 17711 (46 5612) z júla 2022

139961

EUROPEAN STANDARD

EN 17711

NORME EUROPÉENNE

EUROPÄISCHE NORM

November 2024

ICS 65.080

Supersedes CEN/TS 17711:2022

English Version

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.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of 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 United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

EN 17711:2024 (E)

Contents	Page
European foreword.....	5
Introduction	6
1 Scope	7
2 Normative references	7
3 Terms and definitions	8
4 Principle	8
4.1 General.....	8
4.2 Primary enrichment in a liquid selective medium	8
4.3 Secondary enrichment in a liquid selective medium.....	9
4.4 Isolation and identification	9
4.5 Confirmation.....	9
5 Culture media and reagents	9
5.1 Enrichment medium: alkaline saline peptone water (ASPW)	9
5.2 Solid selective isolation media.....	9
5.2.1 First medium: thiosulphate citrate bile and sucrose agar (TCBS) medium.....	9
5.2.2 Second medium.....	10
5.3 Saline nutrient agar (SNA).....	10
5.4 Reagent for detection of oxidase	10
5.5 Reagent for biochemical tests.....	10
5.5.1 L-lysine decarboxylase saline medium (LDC).....	10
5.5.2 Arginine dihydrolase saline medium (ADH)	10
5.5.3 Reagent for detection of β -galactosidase	10
5.5.4 Saline medium for detection of indole	10
5.5.5 Saline peptone water	10
5.5.6 Sodium chloride solution	10
5.5.7 Toluene, p.a.	11
5.5.8 Sterile mineral oil, p.a.	11
6 Equipment and consumables	11
7 Sampling	11
8 Preparation of the test sample	11
9 Procedure (see Figure A.1)	12
9.1 Test portion and initial suspension.....	12
9.2 Primary selective enrichment.....	12
9.3 Secondary selective enrichment.....	13
9.4 Isolation and identification	13
9.5 Confirmation.....	14
9.5.1 General.....	14
9.5.2 Selection of colonies for confirmation and preparation of pure cultures	14
9.5.3 Tests for presumptive identification	14
9.5.4 Biochemical confirmation.....	15
10 Expression of results	17

11	Performance characteristics of the method	17
11.1	Sensitivity	17
11.2	Specificity	17
11.3	LOD50	17
11.4	Precision	17
12	Test report	17
Annex A (normative)	Diagram of procedure	19
Annex B (normative)	Composition and preparation of culture media and reagents	20
B.1	General	20
B.2	Water	20
B.3	Alkaline saline peptone water (ASPW)	20
B.4	Thiosulfate citrate bile and sucrose agar (TCBS)	21
B.5	Saline nutrient agar (SNA)	21
B.6	Reagent for detection of oxidase	22
B.7	L-lysine decarboxylase saline medium (LDC)	22
B.8	Arginine dihydrolase saline medium (ADH)	23
B.9	Detection of β-galactosidase	23
B.10	Saline medium for detection of indole	24
B.11	Saline peptone water	25
B.12	Sodium chloride solution	25
B.13	Tris acetate EDTA (TAE) buffer	25
Annex C (informative)	Conventional PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin (tdh) and thermostable direct related haemolysin (trh) genes, <i>Vibrio cholerae</i> and <i>Vibrio vulnificus</i>	26
C.1	General	26
C.2	Equipment	26
C.3	DNA extraction	27
C.4	Procedure conventional PCR	27
C.5	Primers and probes	28
C.6	Control material — conventional PCR	30
Annex D (informative)	Real-time PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin gene (tdh) and <i>Vibrio vulnificus</i>	32
D.1	General	32
D.2	Equipment	32
D.3	DNA extraction	32
D.4	Real-time PCR	33
D.5	<i>Vibrio parahaemolyticus</i> — primers and hydrolysis probes	33
D.6	Thermostable direct haemolysin (tdh) gene <i>Vibrio parahaemolyticus</i> — primers and hydrolysis probes	33

EN 17711:2024 (E)

D.7	<i>Vibrio vulnificus</i> — primers and hydrolysis probes	34
D.8	Cycling parameters.....	34
D.9	Control material — real-time PCR	34
	Annex E (informative) Repeatability and reproducibility data.....	35
E.1	General.....	35
E.2	Samples.....	35
E.3	Procedure.....	36
E.4	Replicate determination.....	36
E.5	Results.....	36
	Annex ZA (informative) Relationship of this European Standard and the essential requirements of Regulation (EU) 2019/1009 making available on the market of EU fertilising products aimed to be covered.....	38
	Bibliography.....	39

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.

EN 17711:2024 (E)**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.

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 formulations¹, 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 11133:2014², *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)*

koniec náhľadu – text ďalej pokračuje v platenej verzii STN

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