SLOVENSKI STANDARD
SIST EN 17715:2025

01-februar-2025

Nadomešča:
SIST-TS CEN/TS 17715:2023

Rastlinski biostimulanti - Ugotavljanje prisotnosti Shigella spp.

Plant biostimulants - Detection of Shigella spp.

Pflanzen-Biostimulanzien - Nachweis von Shigella spp.

Biostimulants des végétaux - Recherche de Shigella spp.

Ta slovenski standard je istoveten z: **EN 17715:2024**

ICS:

65.080	Gnojila	Fertilizers

SIST EN 17715:2025 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 17715

November 2024

ICS 65.080

Supersedes CEN/TS 17715:2022

English Version

Plant biostimulants - Detection of *Shigella* spp.

Biostimulants des végétaux - Recherche de *Shigella* spp.

Pflanzen-Biostimulanzien - Nachweis von *Shigella* spp.

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

This European Standard was corrected and reissued by the CEN-CENELEC Management Centre on 11 December 2024.

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

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

Ref. No. EN 17715:2024 E

Contents

Page

European foreword

This document (EN 17715:2024) 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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.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 17715:2022.

EN 17715:2024 includes the following significant technical changes with respect to CEN/TS 17715:2022:

— the introduction has been updated and Table 1 has been removed;

— Clause 2, Normative references, has been updated;

— Annex B and Annex C have been revised;

— Annex D on the interlaboratory study has been added;

— Annex ZA has been added.

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.

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 provides a method for verifying that the pathogen *Shigella* spp. is not present in microbial plant biostimulants.

The detection method for *Shigella* pathogens is not sensitive and quantification is rarely performed. Detection is usually performed using an enrichment medium followed by subculturing onto a variety of selective media.

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 percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest percentage in the blend, the European Standard for the highest percentage 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 17708:2024, *Plant biostimulants — Preparation of sample for microbial analysis*

EN ISO 21567:2004, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Shigella spp. (ISO 21567:2004)*

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

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

3 Terms and definitions

For the purposes of this document, the terms and definitions apply.

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

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

3.1
***Shigella* 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

[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 1 kg/kg of plant biostimulant from seaweed.
[2] As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.

3.2
detection of *Shigella* spp.
determination of the presence or absence of these microorganisms in a particular mass of product, when tests are carried out in accordance with this document

3.3
interlaboratory study
study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters

Note 1 to entry: The aim of an interlaboratory study is to determine the variability of the results obtained in different laboratories using identical samples.

[SOURCE: ISO 16140-1:2016, 2.33 [2]]

3.4
sensitivity
number of samples found positive divided by the number of samples tested at a given level of contamination

Note 1 to entry: The results are thus dependent on the level of contamination of the sample.

3.5
specificity
number of samples found negative divided by the number of blank samples tested

3.6
positive predictive value
PPV
ratio of positive samples identified as positive to all those with positive test results (including samples that were incorrectly identified as positive)

3.7
negative predictive value
NPV
ratio of negative samples identified as negative to all those with negative test results (including samples that were incorrectly identified as negative)

4 Principle

4.1 General

Detection of *Shigella* spp. shall be conducted in accordance with the sections specified in EN ISO 21567:2004 and with the following four successive stages (according to Annex A, Figure A.1).

4.2 Enrichment in selective liquid medium

A test portion shall be inoculated with Shigella broth (B.2.1) containing 0,5 µg/ml of novobiocin, then incubated anaerobically at 41,5 °C ± 1°C for 16 h to 20 h.

4.3 Plating out and identification of colonies

From the enrichment culture obtained, three selective differential media shall be inoculated: MacConkey agar with low selectivity; XLD agar with moderate selectivity; Hektoen enteric agar with the greatest selectivity. All shall be incubated at 37 °C ± 1°C for 20 h to 24 h.

4.4 Biochemical and serological confirmation

Typical and suspect colonies shall be selected from each of the three selective agars. The colonies shall be purified on nutrient agar, then biochemical and serological characterizations shall be carried out using the tests described.

5 Culture media, reagents and antisera

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

All media, reagents and antisera that shall be used are specified in Annex B.

Commercially available dehydrated media should give more consistent results than media prepared from their component parts in the laboratory. Follow the manufacturer's instructions exactly, as small changes in preparation can significantly change the performance of selective media. Excessive heating of the selective agars used in this document by autoclaving, storage and then re-heating for use can result in loss of selectivity.

6 Apparatus and glassware

Disposable equipment is an acceptable alternative to re-usable glassware if it has suitable specifications.

Usual microbiological laboratory equipment according to EN 17708:2024 shall be used.

7 Sampling

Sampling is not part of the method specified in this document (the specific European Standard dealing with the product concerned EN 17702-1:2024 shall be used).

The laboratory shall receive a sample which is representative and has not been damaged or changed during transport or storage.

8 Procedure

8.1 General

The presence or absence of *Shigella* pathogens in at least 25 g or 25 ml of the product under test shall be evaluated.

8.2 Test portion

The appropriate part of EN 17708:2024 shall be used.

8.3 Enrichment

The appropriate part of EN 17708:2024 shall be used.

The incubation shall be under anaerobic conditions at 41,5 °C ± 1 °C for 16 h to 20 h.

8.4 Plating out and colony selection

8.4.1 Using the culture obtained in 8.3, gently mix the contents by hand and allow the larger particles to settle.

Inoculate, by means of a loop, the surface of the following selective agars to obtain well-isolated colonies: MacConkey agar (B.3.1) with low selectivity; XLD agar (B.3.2) with moderate selectivity; and Hektoen enteric agar (B.3.3) with a greater selectivity.

8.4.2 Incubate the plates at 37 °C ± 1 °C for 20 h to 24 h.

The appearance of different *Shigella* species can vary on the same selective agar. The morphology and colour of *Shigella* colonies on the different selective agar used shall be evaluated according to Annex C.

Mark any typical or suspect colonies found on each plate.

If no typical colonies are seen and the growth of the other microorganisms is weak, re-incubate the plates for a further 24 h. Examine them again for the typical *Shigella* colonies.

Carry out the confirmation procedure described in 8.5.

8.5 Confirmation of colonies

8.5.1 General

Identification kits (currently commercially available) that have been proven by the user to be reliable for the identification of the different species of *Shigella* may be used. Follow the manufacturer's instructions precisely.

For confirmation, sub-culture, from each dish of each selective medium (see 8.4), five marked typical or suspect colonies.

If on one dish there are fewer than five typical or suspect colonies, take all the marked colonies for confirmation.

Use pure cultures for biochemical and serological confirmation.

8.5.2 Purification of colonies

Streak the selected colonies onto the surface of nutrient agar plates (B.4) or of the same medium (B.3) so as to gain well-isolated colonies. Incubate the plates at 37 °C ± 1 °C for 18 h to 24 h.

If the cultures are not pure, sub-culture the suspect colony onto a further plate and incubate at 37 °C ± 1 °C for 18 h to 24 h to obtain a pure culture.

Shigella sonnei can give two colony types on the same agar plate: a smooth round domed colony (phase 1), and a flat irregular colony with a mat surface (phase 2).

The most characteristic colony from each selective agar plate may be tested first. If positive, other colonies shall not be tested. If negative, progress through the other selected colonies until either all are negative or a positive is found.

8.5.3 Biochemical confirmation

8.5.3.1 General

By means of an inoculation needle, inoculate the media specified in 8.5.3.2 to 8.5.3.9 respectively with each of the cultures selected in 8.5.1 and/or 8.5.2 and record all the results.

If commercially available, consumables (e.g. miniaturized strips) allow to biochemically identify the strains of *Shigella* spp., they may be used instead of the tests from 8.5.3.3 to 8.5.3.9.

8.5.3.2 Triple sugar iron agar (TSI slopes) (B.5)

Stab the butt and streak the agar slope.

Incubate at 37 °C ± 1 °C for 18 h to 24 h.

Interpret the changes in the medium as shown in Table 1.

Table 1 — Interpretation of triple sugar iron agar test

Area of slope	Appearance	Indication
Butt	Yellow Red or unchanged Black Bubbles or cracks	Glucose fermented: positive Glucose not fermented: negative Formation of hydrogen sulfide: positive Gas formation
Slant surface	Yellow Red or unchanged	Lactose and/or sucrose utilized: positive Lactose and sucrose not utilized: negative
Typical *Shigella* spp. cultures show a yellow butt (acid formation) and no gas bubbles, there is no change in the colour of the slant (no utilization of lactose or sucrose) and no hydrogen sulfide production (see Table 2).		

8.5.3.3 Semi-solid nutrient agar for motility tests (B.6)

Stab the semi-solid nutrient agar with a colony using an inoculation needle.

Incubate the tubes at 37 °C ± 1 °C for 18 h to 24 h.

Examine the line of inoculation for spreading growth. Non-motile microorganisms will give a discrete line; motile strains will give diffuse growth away from the inoculum line.

NOTE All *Shigella* species are non-motile.

8.5.3.4 Urea agar (B.7)

Streak the agar surface.

Incubate at 37 °C ± 1 °C for 18 to 24 h and examine at intervals.

If urea is hydrolysed, a rose-pink to deep cerise colour develops from the release of ammonia by the decomposition of the urea with a change in the colour of the pH indicator. There is no change in colour of the agar with a negative reaction.

NOTE *Shigella* species do not hydrolyse urea.

8.5.3.5 L-Lysine decarboxylase medium (B.8)

Inoculate below the surface of the liquid broth. Incubate at 37 °C ± 1 °C for 18 to 24 h.

Turbidity and a purple colour after incubation indicate a positive reaction; yellow indicates a negative result.

Shigella species do not decarboxylate lysine.

NOTE The use of a paraffin overlay in the tubes can help to ensure anaerobic conditions.