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Rastlinski biostimulanti - Ugotavljanje prisotnosti Staphylococcus aureus

Plant biostimulants - Detection of Staphylococcus aureus

Pflanzen-Biostimulanzien - Nachweis von Staphylococcus aureus

Biostimulants des végétaux - Recherche de Staphylococcus aureus

Ta slovenski standard je istoveten z: EN 17712:2024

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ICS: 65.080 Gnojila Fertilizers

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Plant biostimulants - Detection of *Staphylococcus aureus*

Biostimulants des végétaux - Recherche de
Staphylococcus aureus

Pflanzen-Biostimulanzien - Nachweis von
Staphylococcus aureus

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

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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.

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

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

Contents	Page
European foreword	4
Introduction	6
1 Scope	7
2 Normative references	7
3 Terms and definitions	7
4 Principle	8
5 Diluents and media	8
5.1 General	8
5.2 Modified Giolitti-Cantoni broth	8
5.3 Baird-Parker RPF agar	9
6 Apparatus and glassware	9
7 Sampling	9
8 Preparation of test sample	10
8.1 General	10
8.2 Solid formulations	10
8.3 Liquid formulations	10
9 Procedure	10
9.1 Enrichment in Giolitti-Cantoni broth	10
9.2 Isolation on Baird-Parker RPF agar	10
9.3 Selection of plates and interpretation	11
10 Expression of results	11
11 Precision	11
12 Test report	11
13 Performance characteristics of the method	12
13.1 Interlaboratory studies	12
13.2 Sensitivity	12
13.3 Specificity	12
13.4 Positive predictive value (PPV)	12
13.5 Negative predictive value (NPV)	12
Annex A (normative) Composition and preparation of culture media and reagents	13
A.1 General	13
A.2 Baird-Parker Rabbit Plasma Fibrinogen (RPF) agar	13
A.3 Modified Giolitti-Cantoni broth	14
Annex B (informative) Repeatability and reproducibility of the method	15
B.1 Materials used in the interlaboratory comparison study	15
B.2 Interlaboratory comparison results	16
B.3 Contingency table analysis	17
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	18

Bibliography 19

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[SIST EN 17712:2025](https://standards.iteh.ai/catalog/standards/sist/caaf57c2-8607-4c4d-bb4f-e6af41605c7e/sist-en-17712-2025)

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EN 17712:2024 (E)**European foreword**

This document (EN 17712:2024) 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 17712:2022.

EN 17712:2024 includes the following significant technical changes with respect to CEN/TS 17712:2022:

- the European foreword, Introduction and Scope have been updated for consistency with other standards;
- normative references have been updated;
- in Clause 3, the definitions have been revised;
- in Clause 7 and Clause 8, the sampling instructions have been replaced by the relevant normative reference;
- in Clause 8, the sample preparation instructions have been updated in line with comments received;
- in Clause 9, the procedure has been clarified and references to the media and apparatus required have been added;
- a new Clause 10 has been added to specify how the results of the test should be expressed;
- in Clause 12, new elements have been added to the test report requirements;
- Annexes A and B have been revised for consistency with other standards;
- 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.

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EN 17712:2024 (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.

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1 Scope

This document specifies a method to verify that the pathogen *Staphylococcus aureus* is absent from microbial plant biostimulants. The method is based on the enumeration of coagulase-positive staphylococci in a sample by counting of colonies obtained on a solid medium (Baird-Parker medium) after aerobic incubation at $36\text{ °C} \pm 2\text{ °C}$.

This document is applicable to all formulations of microbial plant biostimulants in liquid or solid form. This document is not applicable to other fertilizing products.

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

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

EN 17708:2024, *Plant biostimulants — Preparation of sample for microbial analysis*

EN 17724:2024, *Plant biostimulants — Terminology*

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:

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

3.1

coagulase-positive staphylococci

bacteria that form typical and/or atypical colonies on the surface of Baird-Parker agar and show a positive coagulase reaction or a specific rabbit plasma reaction on Rabbit Plasma Fibrinogen (RPF) agar

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.

EN 17712:2024 (E)**3.2*****Staphylococcus aureus***

bacterium which forms colonies fitting the description of the species on Baird-Parker RPF agar after incubation for 24 h at a temperature of $36\text{ °C} \pm 2\text{ °C}$ under aerobic conditions

Note 1 to entry: *S. aureus* is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when visualized under a microscope, and has round, usually golden-yellow colonies, often with haemolysis, when grown on selective blood agar plates.

Note 2 to entry: Typical *S. aureus* colonies present a white (almost opaque) halo surrounding the colony due to the coagulation of plasma, forming fibrinogen from fibrin.

Note 3 to entry: *S. aureus* colony description:

- circular;
- convex;
- entire margin;
- grey to black (due to the reduction of potassium tellurite to telluride), with a clear zone around the centre of the colony, comparable to a halo.

Note 4 to entry: Colony size varies between 1 mm and 2 mm in diameter.

4 Principle

From a representative sample of the microbial plant biostimulant, an initial suspension shall be prepared with the modified Giolitti-Cantoni broth. This selective enrichment medium shall be incubated under anaerobic conditions at $36\text{ °C} \pm 2\text{ °C}$ for 24 h and 48 h. After that, the presence of presumptive coagulase-positive staphylococci (3.1) is indicated by the reduction of potassium tellurite.

The solid selective culture medium (Baird-Parker RPF agar) shall then be inoculated with the enrichment medium with presumptive coagulase-positive staphylococci. Baird-Parker RPF agar plates shall be incubated at $36\text{ °C} \pm 2\text{ °C}$ and examined after 24 h (and 48 h if necessary) for the presence of typical *S. aureus* colonies (3.2).

The result is given as the presence or absence of *S. aureus* in the 25 g or 25 ml of microbial plant biostimulant sample.

5 Diluents and media**5.1 General**

For current laboratory practice, EN ISO 7218:2024 shall be followed. Performance testing of culture media is recommended to be done in accordance with standards comparable to EN ISO 11133:2014²[2].

Composition of culture media and reagents and their preparation are described in Annex A (normative).

5.2 Modified Giolitti-Cantoni broth

The modified Giolitti-Cantoni broth is used as the selective enrichment medium in this method. This liquid medium shall be prepared in accordance with Annex A.

² As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020