SLOVENSKI STANDARD
oSIST prEN 13040-2:2024

01-julij-2024

Izboljševalci tal in rastni substrati - Priprava vzorcev - 2. del: Priprava vzorcev za mikrobiološke preskuse

Soil improvers and growing media - Sample preparation - Part 2: Sample preparation for microbiological examination

Bodenverbesserungsmittel und Kultursubstrate - Probenvorbereitung - Teil 2: Probenvorbereitung für mikrobiologische Untersuchungen

Amendements du sol et supports de culture - Préparation des échantillons - Partie 2 : Préparation des échantillons pour l'examen microbiologique

Ta slovenski standard je istoveten z: **prEN 13040-2**

ICS:

65.080 Gnojila Fertilizers

oSIST prEN 13040-2:2024 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 13040-2

June 2024

ICS 65.080

English Version

Soil improvers and growing media - Sample preparation - Part 2: Sample preparation for microbiological examination

Bodenverbesserungsmittel und Kultursubstrate - Probenvorbereitung - Teil 2: Probenvorbereitung für mikrobiologische Untersuchungen

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 223.

If this draft becomes a European Standard, 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.

This draft European Standard was established by CEN 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.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

This document (prEN 13040-2:2024) 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 currently submitted the CEN Enquiry.

This document has been prepared under a standardization request addressed to CEN by the European Commission.

Introduction

This document describes the general requirements for the preparation of samples and initial suspensions prior to microbiological examination of soil improvers and growing media.

Any special diluents or practices required for particular materials or microorganisms in specific standard methods take priority over the general requirements listed in this document.

This document is one of the series of standards as listed below:

— prEN 13040-1, *Soil improvers and growing media — Sample preparation — Part 1: Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory bulk density*;

— prEN 13040-2, *Soil improvers and growing media — Sample preparation — Part 2: Sample preparation for microbiological examination*.

WARNING — In order to safeguard the health of laboratory personnel, it is essential that the procedures described in this document are only undertaken in properly equipped laboratories, under the supervision of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document specifies the general requirements for the preparation of samples and initial suspensions prior to microbiological examinations of soil improvers and growing media. This method is intended especially for sample preparation prior to microbiological examinations of e.g. *E. coli*, *Salmonella* spp. and *Enterococcaceae*.

Because of the large variety of soil improvers and growing media, this method might not be appropriate in every detail for certain materials. This method might not be appropriate in every detail for certain products. In this case, different methods which are specific to these products can be used if necessary, for justified technical reasons.

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, *Soil improvers and growing media — Sampling*

CEN/TS 17732, *Soil improvers and growing media — Terminology*

ISO 7218,[1] *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12579, CEN/TS 17732 and the following apply.

3.1
laboratory sample
in relation to chemical and physical testing, a final sample intended for laboratory testing and in relation to microbiological testing, each separate segment sample intended for laboratory testing

3.2
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 and observations are actually carried out

Note 1 to entry: Sometimes a preparation of the laboratory sample (3.1) s required before the test portion is taken out, but this is uncommon in microbiological examinations.

3.3
test sample
sample prepared from the laboratory sample (3.1) and from which test portions (3.2) will be taken

Note 1 to entry: Preparation of the laboratory sample (3.1) before taking out the test portion (3.2) is rarely used in microbiological examinations.

[1] In preparation.

3.4
initial suspension
primary dilution obtained after the test portion (3.2) has been treated with, normally, a nine-fold quantity of diluent

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

3.5
further dilution
suspension or solution obtained by mixing a measured volume of the initial suspension (3.4) with an x-fold volume of diluent and by repeating this operation with further dilutions until a dilution series is obtained that is suitable for the inoculation of culture media

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

4 Principle

Sample preparation prior to microbiologal examination consists of two consecutive steps, for which the second step depends on the intent of the subsequent microbiological examination, meaning enumeration or detection.

1) Take the test portion (3.2) out of the laboratory sample (3.1):

2) Preparation:

   a) **For enumeration**

   Preparation of an initial suspension in a way to obtain an as uniform as possible distribution of microorganisms contained in the test portion. Preparation, if necessary, of further dilutions in order to reduce the number of microorganisms per unit volume to allow counting of colonies after incubation.

   b) **For detection**

   Preparation of a first enrichment in a way to obtain an as uniform as possible distribution of microorganisms contained in the test portion.

To restrict the enumeration range to a given optimal interval, or if high numbers of microorganisms are expected, only those (decimal) dilutions that are necessary may be inoculated to perform the enumeration comparable to calculation in e.g. ISO 7218[1].

5 Reagents

Follow current laboratory practices in accordance with standards comparable to ISO 7218[1]. The composition of culture media and reagents and their preparation are specified in Annex A. For uniformity of results, in the preparation of media use either dehydrated complete medium or use constituents of uniform quality and chemicals of recognized analytical grade. For performance testing of culture media, follow the procedures in accordance with standards comparable to EN ISO 11133 and Annex A.

6 Apparatus and equipment

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications. The usual microbiological laboratory equipment (see ISO 7218[1]) and, in particular, the following shall be used.

6.1 Apparatus for dry sterilization (oven) or **wet sterilization (autoclave)**.

6.2 Equipment for size reduction; apparatuses (aseptic) may include:

— scissors;

— tweezers;

— straight scalpels;

— knives;

— hammers (used with a sterile bag around the sample);

— saws;

— spatulas;

— mortar and pestle.

6.3 Homogenization devices; apparatuses (aseptic) may include:

— a magnetic stirrer;

— orbital shaker, with bottles or plastic bags (glass beads may be used in the case of viscous or thick materials);

— peristaltic blender (paddle blender), with sterile bags, preferable with a filter;

— vortex mixer (for dilutions).

6.4 Other laboratory equipment may include:

— pipettes or pipettor and sterile tips, to dispense 0,1 ml to 1 ml with tips (with large opening if necessary);

— scales and gravimetric dilution controllers, capable of weighing to 1 % of the mass;

— sterile bowls, wide-mouth containers or plastic bags.

7 Transportation and storage of samples

The laboratory sample shall be transported and stored without compaction or any other treatment which may irreversibly alter its moisture content, particle size, packing characteristics or any feature which affects density.

The laboratory samples, as submitted to the laboratory, shall be stored so that they shall not undergo any further decomposition, physical damage, hydration or dehydration. Recommended storage should be at 5 °C ± 3 °C, but not frozen.

NOTE It is recommended to examine the samples within 72 h after reception as the microbiome might change over time.

8 Procedure

8.1 General

The requirements for the different general categories of materials subjected to microbiological examination are given in this section.

8.2 Preparation of the test portion

The types of packaged materials sent to the laboratory can be in flexible packaging. This is to be removed or opened aseptically with scissors, knives or scalpels (6.2).

All operations before and after opening the packaged material shall be carried out under aseptic conditions, to avoid any external contamination.

NOTE There is no need to disinfect the packaging if the contents may be removed aseptically after opening without any risk of external contamination.

Pre-processing may be required before the test portion (3.2) is taken. These include:

— **For pre-shaped materials:** The test portion (3.2) is taken directly from the material, without "reconstitution" by addition of water.

— **For solid rigid materials:** Solid materials shall be reduced in size to obtain a representative test sample (3.3) under aseptic conditions (6.1). The dimension of the pieces shall not exceed approximately 2 cm in all dimensions.

Remove any incidental pieces (e.g. stones) with a sterile spoon or tweezers (6.2). Then homogenize for 1 min to 3 min (paddle blender), or approximately 15 minutes (orbital shaker and magnetic stirrer) with a homogenization device (6.3), or until a homogeneous suspension is obtained.

Table B.1 provides an overview of exemplary materials and recommended equipment as well as a description of the operating procedure for sample preparation.

8.3 Preparation of the initial suspension (primary dilution)

Weigh at least 10 g (m) or measure at least 10 ml (V) of the test portion (3.2) in a sterile container or plastic bag, unless stated differently in the specific standard for the microorganism to be examined, or for combined weighting for several microorganisms. The tolerance for a measured sample weight or volume should be within ± 5 % and the volume of diluent within ± 2 %.

The final volume shall be 10 times the volume of the test portion.

For the combined examination of Salmonella and other microorganisms, e.g. weigh 26 g into a sterile container and add 234 ml buffered peptone water or double-buffered peptone water. Homogenize the solution and remove 10 ml for enumeration essays. (i.e. 250 ml for detection of Salmonella). In this case, rapid processing of the initial suspension is necessary.

The amount of sample shall adequately represent the material to be tested. In case of examination of very coarse inhomogeneous material, the sample quantity should be increased.

To avoid damage to microorganisms by sudden temperature changes, the temperature of all diluents shall be approximately equal to the ambient temperature of the laboratory, unless otherwise specified for particular materials.

For products that swell in water, the test portion-to-diluent ratio should be increased successively (e.g. 1 to 20, 1 to 50, as appropriate) until a suitable suspension is obtained. Record the use of additional