PD CEN/TS 17781:2022

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Organic, organo-mineral and inorganic fertilizers — Detection of *Escherichia coli*

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Published by BSI Standards Limited 2022

ISBN 978 0 539 17961 3

ICS 65.080

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This Published Document was published under the authority of the Standards Policy and Strategy Committee on 31 May 2022.

Amendments/corrigenda issued since publication

Date	Text affected

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TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 17781

April 2022

ICS 65.080

English Version

Organic, organo-mineral and inorganic fertilizers - Detection of *Escherichia coli*

Engrais organiques, organo-minéraux et inorganiques - Recherche des *Escherichia coli*

Organische, organisch-mineralische und anorganische Düngemittel - Nachweis von *Escherichia coli*

This Technical Specification (CEN/TS) was approved by CEN on 13 March 2022 for provisional application.

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European foreword

This document (CEN/TS 17781:2022) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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Introduction

This document describes a method for the detection and enumeration of *Escherichia coli* in fertilizers of the following Product Function Categories (PFCs) of EU fertilizing products, as described in the Regulation (EU) 2019/1009 [1]:

— PFC 1(A): Organic fertilizer;

— PFC 1(B): Organo-mineral fertilizer;

— PFC 1(C): Inorganic fertilizer, which contains more than 1 % by mass of organic carbon, other than organic carbon from chelating or complexing agents, nitrification inhibitors, denitrification inhibitors or urease inhibitors, coating agents, urea or calcium cyanamide. The present method was validated on products known as present on the market in April 2021 and conform to Regulation (EU) 2019/1009 [1] that are inorganic fertilizers with more than 1 % of organic carbon such as struvite with low level of organic matter. In case that other products would be developed having other physical and chemical characteristics, it might become necessary to develop different methods to correctly account for pathogenic microorganisms they might contain.

This methodology has been developed to detect and enumerate *Escherichia coli* in organic, organo-mineral and inorganic fertilizers in order to be able to control certain hygienic requirements in the Regulation (EU) 2019/1009 [1].

Escherichia coli is a Gram negative bacterium with a faecal origin. Consequently, it can be used as an indicator of faecal contamination. It can also be used to monitor the effectiveness of pasteurization or disinfection treatments but it is comparatively sensitive (to heat, high pH) and therefore cannot reflect the behaviour of all pathogens in fertilizers.

Because of the large variety of fertilizers, this method might not be appropriate in every detail for certain products. In this case, different methods which are specific to these products may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this method as far as possible.

Mineral components in fertilizers can have a negative impact on the survivability of microorganisms when they go into solution. In addition to an unfavourable shift in the pH value, the products can have a strong osmotic effect or be toxic to cells themselves (e.g. copper). Therefore, it can be necessary to test the inhibitory effect of the fertilizers to be investigated in a pre-test.

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1 Scope

This document is applicable to fertilizing products, which are classified as PFC 1(A) and PFC 1(B) or the PFC 1(A) and PFC 1(B) component in PFC 7 of Regulation (EU) 2019/1009 [1]. However, the present method was not validated for blends.

This document specifies a colony-count technique at 44 °C on a solid medium containing a chromogenic ingredient for the detection of the enzyme β-glucuronidase. The method is based on ISO 16649-2 [4].

Strains of *Escherichia coli* which do not grow at 44 °C and, in particular, those that are β-glucuronidase negative, such as *Escherichia coli* O157, will not be detected. Detected microorganisms are presumptively determined β-glucuronidase-positive *Escherichia coli*, since some Enterobacteriaceae, in particular Shigella and Salmonella, can also show β-glucuronidase activity at 44 °C.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
laboratory sample
sample intended for laboratory inspection or testing

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

3.3
test portion
quantity of material taken from the test sample (or if both are the same, from the laboratory sample) and on which the test is carried out

3.4
glucuronidase-positive presumptive *Escherichia coli*
bacteria which at 44 °C form typical blue colony on Tryptone-Bile-X-glucuronide (TBX) medium under the conditions specified in this method

[SOURCE: ISO 16649-2:2001, 3.1, modified – The reference to ISO 16649-2 has been replaced with a reference to this document and the term name has been modified.]

3.5
confirmed β-glucuronidase-positive *Escherichia coli*
β-glucuronidase-positive presumptive *Escherichia coli* (3.4) showing a positive indole reaction in tryptophan broth under the conditions specified in this method

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