PD CEN/TR 16193:2013

BSI Standards Publication

Sludge, treated biowaste and soil — Detection and enumeration of Escherichia coli

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National foreword

This Published Document is the UK implementation of CEN/TR 16193:2013.

This technical report contains details of three methods evaluated for their suitability for 'horizontal' application for sludge, treated biowaste and soil. The UK committee believes that these methods are not fit for their intended purpose and are not appropriate to samples of all such materials, as the sample matrices are not sufficiently similar.

It should be noted that in merging three separate drafts to achieve the final text, incompatibilities and conflicting interpretation have been introduced:

— the document does not adhere to a recognised definition of *E. coli* as target organism (see SCA methods referred to below);

— the inter laboratory validation undertaken (as found in Tables 4, 9 and 14) is considered by UK technical experts as demonstrating poor reproducibility;

— soil matrices were not included in the validation.

The recommended methods for UK laboratories intending to analyze waste-water sludge can be found in 'The Microbiology of Sewage Sludge: Part 3 (2003) — Methods for the isolation and enumeration of *Escherichia coli,* including verocytotoxigenic *Escherichia coli*' published by the Environment Agency Standing Committee of Analysts (SCA), in the series 'Methods for the examination of water and associated materials'.

SCA methods are also considered suitable for determining *E. coli* in some treated biowaste applications such as compost, under PAS 100:2005, and digestates, under PAS 110:2010. BS ISO 16649-2:2001 provides a method suitable for enumeration of *E. coli* in food and animal feeding stuffs. These methods might also be applicable to soil but this would need to be verified using the approach described in DD ENV ISO/TR 13843:2001, *Water Quality — Guidance on validation of Microbiological Methods*.

The UK participation in its preparation was entrusted to Technical Committee H/-/4, Environmental testing programmes.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Sludge, treated biowaste and soil - Detection and enumeration of Escherichia coli

Boue, biodéchet traité et sol - Recherche et dénombrement des Escherichia coli

Schlamm, behandelter Bioabfall und Boden - Nachweis und Zählung von Escherichia coli

This Technical Report was approved by CEN on 1 March 2011. It has been drawn up by the Technical Committee CEN/TC 400.

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Foreword

This document (CEN/TR 16193:2013) has been prepared by Technical Committee CEN/TC 400 “Project Committee - Horizontal standards in in the fields of sludge, biowaste and soil”, the secretariat of which is held by DIN.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

This document is part of a modular horizontal approach in which this document belongs to the analytical step.

The preparation of this document by CEN is based on a mandate by the European Commission (Mandate M/330). The mandate considers standards on sampling and analytical methods for hygienic and biological parameters as well as inorganic and organic determinants. It was the aim of the mandate to develop standards that are applicable to sludge, treated biowaste and soil and lead to equivalent results as far as this is technically feasible.

Until now, test methods determining properties of materials within the environmental area were prepared in Technical Committees (TCs) working on specific products/matrices (soil, waste, sludge etc). However, it is recognised that many steps in test procedures can be used in test procedures for other products/matrices. By careful determination of these steps and selection of specific questions within these steps, elements of the test procedure can be described in a way that can be used for more matrices and materials with certain specifications. This optimisation is in line with the development among end-users of standards. A majority of routine environmental analyses are carried out by institutions and laboratories working under a scope which is not limited to one single environmental matrix but covers a wide variety of matrices. Availability of standards covering more matrices contributes to the optimisation of laboratory procedures and standard maintenance costs, e.g. costs related to accreditation and recognition.

A horizontal modular approach was developed in the project 'Horizontal'. 'Modular' means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole "chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardising the standard “chain”.

The results of the desk study as well as the evaluation and validation studies have been subject to discussions with all parties concerned in the CEN structure during the development by project 'Horizontal'. The results of these consultations with interested parties in the CEN structure have been presented to and discussed in CEN/TC 400.

This Technical Report contains the most common detection and enumeration methods for the determination of *E. coli* consolidated in one document. The individual methods are specified in the following clauses:

— Clause 6: Method A - Membrane filtration method for quantification;

— Clause 7: Method B - Miniaturised method (Most Probable Number) by inoculation in liquid medium;

— Clause 8: Method C - Macromethod (Most Probable Number) in liquid medium.

Introduction

Escherichia coli is a non-pathogenic, 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 pasteurisation or disinfection treatments but it is comparatively sensitive (to heat, high pH) and cannot therefore reflect the behaviour of all pathogens in these materials.

This Technical Report contains three different methods for the detection and enumeration of *Escherichia coli* which were included in a validation trial in 2007.

The results achieved in this validation trial have been judged differently by experts. Consequently, it was decided by CEN/TC 400 to publish the methods as a Technical Report, aiming for further improvement of the methods and a later publication as European Standard.

Table 1 — Matrices for which the methods described in this Technical Report are applicable and tested in a validation trial

Matrix	Method A	Method B	Method C
Sludge	Mesophilic anaerobic digested sewage sludge	Mesophilic anaerobic digested sewage sludge	Mesophilic anaerobic digested sewage sludge
	Pelletised air dried sludge	Pelletised air dried sludge	Pelletised air-dried sludge
	Digested sewage sludge presscake	Digested sewage sludge presscake	Digested sewage sludge presscake
	Composted sewage sludge	Composted sewage sludge	Composted sewage sludge
Biowaste	Composted biowaste	Composted biowaste	Anaerobic treated biowaste
	Composted green waste	Composted green waste	Composted green waste
	Anaerobic treated biowaste	Anaerobic treated biowaste	Composted biowaste

WARNING — Persons using this Technical Report should be familiar with normal laboratory practice. This Technical Report 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.

WARNING — Samples may contain hazardous and inflammable substances. They may contain pathogens and be liable to biological action. Consequently, it is recommended that these samples be handled with special care. The gases which can be produced by microbiological activity are potentially inflammable and will pressurise sealed bottles. Exploding bottles are likely to result in infectious shrapnel and/or pathogenic aerosols. Glass bottles should be avoided wherever possible. National regulations should be followed with respect to microbiological hazards associated with this method.

IMPORTANT — It is absolutely essential that tests conducted according to this Technical Report be carried out by suitably trained staff.

1 Scope

This Technical Report specifies three methods for the detection and enumeration of *Escherichia coli* in sludge, treated biowaste and soil:

— Method A - Membrane filtration method for quantification (see Clause 6);

— Method B - Miniaturised method (Most Probable Number, MPN) by inoculation in liquid medium (see Clause 7);

— Method C - Macromethod (Most Probable Number) in liquid medium (see Clause 8).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15934, *Sludge, treated biowaste, soil and waste — Calculation of dry matter fraction after determination of dry residue or water content*

EN ISO 9308-3:1998, *Water quality — Detection and enumeration of Escherichia coli and coliform bacteria in surface and wastewater — Part 3: Miniaturized method (Most Probable Number) by inoculation in liquid medium (ISO 9308-3:1998)*

ISO 8199, *Water quality — General guidance on the enumeration of microorganisms by culture*

3 Terms and definitions

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

3.1
Escherichia coli
E.coli
β-D-glucuronidase-positive microorganism growing at an incubation temperature of 44 °C in the specified liquid medium containing 4-methylumbelliferyl-β-D-glucuronide (MUG)

[SOURCE: EN ISO 9308-3:1998]

Note 1 to entry: During growth indole is produced from tryptophan and gas produced from lactose.

3.2
vegetative bacteria
bacteria which are capable of normal growth in broth or on agar media without pre-culture resuscitation

3.3
sub-lethally damaged bacteria
bacteria which have been stressed but not killed by storage or subsequent treatment by, e.g., mesophilic anaerobic digestion, lime stabilisation or composting, and therefore may not be recovered

3.4
resuscitation
recovery to vegetative growth of sub-lethally damaged bacteria previously incapable of growth on agar media