TECHNICAL REPORT

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# CEN/TR 15214-1

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English Version

# Characterization of sludges - Detection and enumeration of Escherichia coli in sludges, soils, soil improvers, growing media and biowastes - Part 1: Membrane filtration method for quantification

Caractérisation des boues - Détection et dénombrement des Escherichia coli dans les boues, les sols, les engrais, les amendements organiques et les biodéchets - Partie 1 : Méthode de quantification par filtration sur membrane

Charakterisierung von Schlämmen - Quantitativer Nachweis von Escherichia coli in Schlämmen, Böden, Düngemitteln und Bodenverbesserern, Kultursubstraten sowie Bioabfällen - Teil 1: Membranfiltrationsverfahren zur quantitativen Bestimmung

This Technical Report was approved by CEN on 3 September 2005. It has been drawn up by the Technical Committee CEN/TC 308.

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## Foreword

This Technical Report (CEN/TR 15214-1:2006) has been prepared by Technical Committee CEN/TC 308 “Characterization of sludges”, the secretariat of which is held by AFNOR.

This Technical Report does not replace any existing CEN method.

The standard is divided into three parts, part 1 gives a membrane filtration for quantification, part 2 gives a miniaturised semi-quantitative MPN method and part 3 gives a semi-quantitative macromethod.

## Introduction

Sludges, soils, soil improvers, growing media and biowastes can contain pathogenic micro-organisms such as *Salmonella* spp. which occur mainly in the intestinal tract of humans and animals and are transmitted through faecal contamination. The use of such contaminated materials in agriculture may cause outbreaks of infection due to the production of contaminated food and animal foodstocks. They may also be transmitted to wild animals. There is a need to monitor the efficacy of the storage and treatment processes to control pathogens such as *Salmonella* spp., and application rates to land.

*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 pasteurization or disinfection treatments but it is comparatively sensitive (to heat, high pH) and cannot therefore reflect the behaviour of all pathogens in these materials.

Suitable quality control procedures, at least those described in ISO 8199, have to be applied.

**WARNING — "Waste and sludge samples can contain hazardous and inflammable substances. They can contain pathogens and be liable to biological action. Consequently, it is recommended that these samples should 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".**

## 1 Scope

This part of the CEN Technical Report specifies a membrane filtration procedure for the quantitative detection, by culture of individual colonies on chromogenic agar media, of *Escherichia coli*. in sludges, soils, soil improvers, growing media and biowastes. This part of the Technical Report is not suitable for materials whose treatment will significantly reduce bacterial levels to less than 10 viable *E. coli* per g wet weight, such as lime addition, drying or pasteurisation. A liquid enrichment and most probable number estimation method may be suited for such purpose.

This membrane filtration method is not appropriate for enumeration and detection of other coliform bacteria without modifications to the chromogenic agar media.

It is suitable to evaluate the log reduction of *E.coli* through treatment, as well as the quality of the end product.

This method is for materials with dry residues less than 20 %. For materials with dry residues greater than 20 % and low numbers of *E. coli*, CEN/TR 15214-2 and CEN/TR 15214-3 should be used.

## 2 Normative references

The following referenced documents are indispensable for the application 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 12880:2000, *Characterisation of Sludges — Determination of dry residue and water content*

ISO 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

## 3 Terms and definitions

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

**3.1**
***Escherichia coli***
*Escherichia coli*, belongs to the family of *Enterobacteriaceae*, is Gram-negative, non-sporulating, rod-shaped, lactose positive and able to grow at 44 °C. Most *E. coli* strains are able to produce indole from tryptophan and are β-glucuronidase-positive

**3.2**
**method definition**
β-glucuronidase-positive and able to hydrolyse 5-bromo-4-chloro-3-indolyl-β-glucuronide (BCIG) when growing on an MLG agar medium at the temperature of 44 °C

**3.3**
**cfu, colony forming unit**
growth of individual bacterial cells into visible colonies on agar media, including on membrane filters overlaying the agar media

**3.4**
**vegetative bacteria**
those bacteria which are capable of normal growth in broth or on agar media without pre-culture resuscitation

**3.5**
**sub-lethally damaged bacteria**
those bacteria which have been stressed but not killed by storage or subsequent treatment by, for example, mesophilic anaerobic digestion, lime stabilisation or composting