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English Version

Characterization of sludges - Detection and enumeration of Salmonella spp. in sludges, soils, soil improvers, growing media and biowastes - Part 2: Liquid enrichment method in selenite-cystine medium followed by Rapport-Vassiliadis for semi-quantitative Most Probable Number (MPN) determination

Caractérisation des boues - Détection et dénombrement de Salmonella spp. dans les boues, les sols, les engrais, les amendements organiques et biodéchets - Partie 2 : Méthode par enrichissement en milieu liquide sélénite-cystine puis en milieu de Rapport-Vassiliadis pour la détermination semi-quantitative par la méthode du Nombre le Plus Probable (NPP)

Quantitativer Nachweis von Salmonella spp. in Schlämmen, Böden, Düngemitteln und Bodenverbesserern, Kultursubstraten sowie Bioabfällen - Teil 2: Flüssiganreicherungsverfahren in Selenit-Cystein-Medium gefolgt durch Rapport-Vassiliadis zur semiquantitativen Bestimmung der höchstwahrscheinlichen Keimzahl (MPN)

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 15215-2:2006) 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 standard.

This Standard is divided in three parts:

- part 1 gives a membrane filtration method

- part 2 is a liquid enrichment method and determination MPN and

- part 3 is a presence/absence method by liquid enrichment.

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 pathogen-contaminated materials in agriculture can cause outbreaks of infection due to the production of contaminated food or animal feedstocks and may also be transmitted to wild animals, consequently, there is a need to monitor rates to land.

Examination for *Salmonellae* should only be carried out in laboratories competent for carrying out work involving pathogens. 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 method describes a method to detect and semi-quantitatively determine *Salmonellae* in sludges, soils, soil improvers, growing media and biowastes in accordance with the requirements of the European Sewage Sludge Regulation Revision of Directive 86/278/EEC (3rd Draft, CEN/TC 308 – doc525).

The fully defined scope will be determined after the proposed validation trials have been agreed and carried out. The method has a limit of detection of approximately 1cfu/g wet weight sample.

NOTE The objective is to cover untreated and treated sludges, soils, soil improvers, growing media, biowastes and associated materials.

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 guide to 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
***Salmonella* spp.**
member of the family of *Enterobacteriaceae*, these are Gram-negative, non-sporulating, rod-shaped bacteria, most of which are motile. They can be distinguished from other genera of the *Enterobacteriaceae* family by biochemical methods and serologically identified by their somatic or flagellar antigens (O and H-antigens)

3.2
method definition
Salmonella spp. capable of being enriched in selenite cystine broth at (36 ± 2) °C followed by growth in Rappaport-Vassiliadis medium at (42 ± 1) °C followed by characteristic growth on SMID/Rambach agar or XLD agar at (36 ± 2) °C (see also 4 and 8.5)

NOTE Some *Salmonella* (e.g. *S. typhi* and *S. paratyphi*) will not be detected.

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 in treatment processes or storage