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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 3: Presence/absence method by liquid  
enrichment in peptone-novobiocin medium followed by Rapport-  
Vassiliadis**

Détection et dénombrement de *Salmonella* spp. dans les  
boues, les sols, les engrais, les amendements organiques  
et les biodéchets - Partie 3: Présence/absence par  
enrichissement en milieu liquide peptone-novobiocine puis  
sur milieu Rapport-Vassiliadis

Quantitativer Nachweis von *Salmonella* spp. in  
Schlämmen, Böden, Düngemitteln und Bodenverbesserern,  
Kultursubstraten sowie Bioabfällen - Teil 3: Verfahren der  
Flüssiganreicherung in Peptonwasser mit Novobiocin  
gefolgt durch Rapport-Vassiliadis zum qualitativen  
Nachweis des Vorkommens

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 15215-3:2006) has been prepared by Technical Committee CEN/TC 308 "Characterisation of sludges", the secretariat of which is held by AFNOR.

This Technical Report does not replace any existing CEN method

This standard is divided into three parts:

- part 1 gives a membrane filtration method
- part 2 is a liquid enrichment method and determination by 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. See CEN/TR 15215-2.

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 specifies a presence/absence procedure to detect *Salmonella* spp using a four-stage presence/absence method in up to 50g (wet weight) sample.

The method has a limit of detection of approximately 10 cfu/50 g wet weight sludge.

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

## 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 peptone water supplemented with Novobiocin and growth in RV medium

### 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

### 3.6

#### **resuscitation**

stimulation to vegetative growth of sub-lethally damaged bacteria previously incapable of growth on agar media

### 3.8

#### **presumptive positives**

isolates which are believed to be *Salmonella* spp., but not yet confirmed