SLOVENSKI STANDARD

SIST-TP CEN/TR 15215-3:2006

01-julij-2006

Karakterizacija blata ï Ugotavljanje prisotnosti in ġtevila Salmonela sp. v blatu, zemljini, izboljġevalcih tal, rastnih substratih in bio-odpadkih - 3. del: Metoda s tekoļ o obogatitvijo v pepton-novobiocin mediju, ki mu sledi metoda po Vassiliadisu za kvalitativno doloļ evanje prisotnosti

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

Charakterisierung von Schlämmen - Quantitativer Nachweis von Salmonella spp. in Schlämmen, Böden, Bodenverbesserungsmitteln, Kultursubstraten sowie Bioabfällen - Teil 3: Verfahren der Flüssiganreicherung in Peptonwasser mit Novobiocin in Kombination mit Rappaport-Vassiliadis-Medium zum qualitativen Nachweis des Vorkommens

Caractérisation des boues - Détection et dénombrement de Salmonella spp. dans les boues, les sols, les amendements du sol, les supports de culture et les biodéchets - Partie 3: Présence/absence par enrichissement en milieu liquide peptone-novobiocine puis sur milieu Rapport-Vassiliadis

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TECHNICAL REPORT

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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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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

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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

3.9
dry residue
the dry mass portion of the sludge obtained after the specified drying process. It is expressed as percent or in grams per kilogram

[EN 12880:2000, 3.1]

4 Principle

This is a presence/absence method including recovery of sub-lethally damaged *Salmonella* spp. Designed to process samples of up to 50 g wet weight. If lower sample quantities are processed, the relationship between the amount of sample and primary recovery medium shall be maintained

The detection of *Salmonella* spp*.* requires four stages.

a) culturing of bacteria in a primary selective medium;

b) enrichment in a secondary selective medium which inhibits the growth of other micro-organisms but promotes that of *Salmonellae* (selective enrichment);

c) preparation of pure cultures by inoculation of two different solid media with subcultures;

d) biochemical and serological identification

5 Apparatus

With the exception of equipment supplied sterile, the glassware shall be sterilised in accordance with the instructions given in ISO 8199.

Usual microbiological laboratory equipment and in particular:

5.1 Wide-mouth glass flasks or beakers for example 125 ml, 200 ml, 500 ml and 2 000 ml.

5.2 Thermostatic incubators regulated at (36 ± 2) °C (gyratory shaking) and (42 ± 1) °C (static).

5.3 Autoclave (Steam sterilizer).

5.4 Refrigerator.

5.5 Sterile plastics culture dishes, with lid of about 90 mm in diameter.

5.6 Sterile graduated pipettes, of nominal capacities 1 and 10 ml.

5.7 Inoculating loop (e.g. platinum-iridium wire), of diameter approximately 3 mm.

5.8 Apparatus for shaking the culture tubes.

5.9 Culture tubes, 25 ml capacity, or equivalent containers.

5.10 Vortex mixer suitable for 25 ml capacity culture tubes or equivalent containers.

5.11 Laboratory spatula.

5.12 pH meter, with temperature compensation and pH measuring cell.

5.13 Membrane filtration equipment.

5.14 Filter membrane, for media sterilisation (0,2 µm cellulose nitrate 47 mm diameter).

5.15 Adjustable micropipettor up to 200 µl capacity.

5.16 Boiling water bath.

6 Sampling and hazards

6.1 Introduction

Take samples of at least 100 g wet weight and deliver them to the laboratory as quickly as possible (within 24 hours). In order to prevent propagation or inactivation of *Salmonella* during transport to the laboratory and subsequent storage, the necessary precautions depending upon the matrix shall be taken.

NOTE Generally chilling the sample to (5 ± 3) °C is recommended.

6.2 General

Samples are liable to ferment particularly if not treated, and may contain pathogenic micro-organisms. It is essential to keep them away from any food or drink, and to protect any cuts. When transporting and handling samples, it is essential that national and international regulations relating to biohazardous samples are followed.

See also the Warning note in the introduction.

6.3 Storage

It is not advisable to store samples in the open laboratory. If samples are to be stored, store them at (5 ± 3) °C for a maximum period of 36 hours.

6.4 Handling

Cleanliness when working is essential. When handling sludge samples, it is necessary to wear gloves, a face and eye protection, sufficient body protection to guard against bottles bursting. The gas evolved is usually flammable, so all equipment in the vicinity shall be flame proof to avoid any source of ignition.

See also the Warning note in the introduction.

7 Reagents, diluents and culture media

To ensure reproducible results, prepare culture media and diluents using either constituents of uniform quality and chemicals of recognised analytical grade, or a dehydrated diluent or complete medium prepared following the manufacturer's instructions. Prepare them with fit for purpose demineralised or distilled water free from substances capable of inhibiting growth under the test conditions. (ISO 8199). If the media are not used immediately, preserve them in the dark at (5 ± 3) °C for up to one month in conditions avoiding any alterations in their composition.

NOTE The use of chemicals of other grades is permissible provided that they are shown to be of equivalent performance in the test.