Soils, sludges and treated bio-wastes — Detection and enumeration of *Escherichia coli* in sludges, soils and organic fertilisers of similar consistency to the matrices validated **—** Part 3: Macromethod (Most Probable Number) in liquid medium

Boden, Schlamm und behandelte Bioabfälle — Quantitativer Nachweis von Escherichia coli aus Schlämmen, Böden, Düngemitteln und Bodenverbesserern, Kultursubstraten sowie Bioabfällen — Teil 3: Makromethode (MPN) in Flüssigmedium

Sols, boues et bio-déchets traités — Détection et dénombrement de Escherichia coli dans les boues, les sols et les fertilisants organiques de consistance similaire aux matrices validées — Partie 3 : Macro-méthode (NPP) par ensemencement en milieu liquide

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Contents

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Foreword

This document has been prepared in the framework of the project Horizontal.

This document is a working document.

The following TC's have been involved in the preparation of the standard: TC 308.

The standard is divided into three parts:

- part 1 describes a membrane filtration method for quantification,
- part 2 describes a miniaturised semi-quantitative MPN method,
- part 3 describes a semi-quantitative MPN macromethod.

This standard is applicable and validated for several types of matrices. The table below indicates which ones.

Material	Validated	Document
Mesophilic anaerobic digested sewage sludge		
Anaerobic treated biowaste		
Pelletised air dried sludge		
Digested sewage sludge presscake		
Composted sewage sludge		
Composted green waste		
Composted biowaste		

Introduction

This document is developed in the framework of the project 'Horizontal'. It is the result of a desk study "Hygienic Parameters Feasibility of Horizontal Standards for *Escherichia coli* and *Salmonella* spp. in sludges, soils, soil improvers, growing media and biowastes" and aims at evaluation of the latest developments in assessing *E. coli* in sludge, soil and organic fertilisers. After discussion with all parties concerned in CEN and selection of a number of test methods described in this study the standard has been developed further as a modular horizontal method and has been validated within the project ”Horizontal”.

Sludges, soils and organic fertilisers 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 feedstocks. 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 8199:2005, 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".

The texts of the chapters 1 to 12 are normative; annexes are normative or informative, as stated in the top lines of the annexes.

1 Scope

This part of the European standard describes a most probable number (MPN) method for the semi-quantitative detection of *Escherichia coli* in sludges, soils and organic fertilisers of similar consistency to the matrices validated. It is suitable to evaluate the log reduction of *E.coli* through treatment as well as the quality of the end product.

This method can be used irrespective of the dry residue content of the test material.

The method has a limit of detection of approximately 10 *E. coli* MPN/g wet weight [ENV ISO 13843].

2 Normative references

These normative references are cited at appropriate places in the text and the publications are listed hereafter. For dated references, only the edition cited applies. For undated references the latest edition of the publication referred to applies (including any amendments).

EN ISO 5667-13:1997, *Water Quality — Sampling — Part 13: Guidance on sampling of sludges from sewage and water treatment works*.

EN 12880:2000, *Characterisation of sludges — Determination of dry residue and water content*.

ISO 8199:2005, *Water quality — General guidance on the enumeration of micro-organisms by culture*.

ENV ISO 13843:2001, *Water quality — Guidance on validation of microbiological methods*.

ISO 5725:1994, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests*.

3 Definitions

For the purposes of this document, 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 bacteria, able to ferment lactose and 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 able to hydrolyse 4-methylumbelliferyl-β-D-glucuronide (MUG) when growing at the incubation temperature of 44 °C in the specified liquid medium. In addition, indole shall be produced from tryptophan and gas produced from lactose

3.3
Most probable number (MPN)
Every tube whose inoculum contains even one viable organism will produce detectable growth or change. The individual tubes of the sample are independent. The essence of the MPN method is to dilute the sample to such a degree that inocula in the tubes will sometimes but not always contain viable organisms. The "outcome", i.e., the number of tubes and the number of tubes with growth at each dilution, will imply an

estimate of the original, undiluted concentration of bacteria in the sample. In order to obtain estimates over a broad range of possible concentrations, microbiologists use serial dilutions incubating tubes at several dilutions. The MPN is the number which makes the observed outcome most probable

3.4
dry residue
the dry mass portion of the material obtained after the specified drying process. It is expressed as percent or in grams per kilogram [EN 12880:2000]

4 Symbols and abbreviations

MPN: Most Probable Number

MUG: 4-methyl-umbelliferyl-β-D-glucuronide

E. coli: *Escherichia coli*

5 Principle

This method is based on that described by Schindler (1991) [2].

a) Preparation of the homogenised sample suspension of the sample in 0.9 % m/V sodium chloride;

b) Serial dilutions of this suspension in the same diluent (from 10^{-1} up to 10^{-7})

c) Transfer of 3 x 1mL out of each dilution step into 3 tubes containing 9 mL Fluorocult™ lauryl sulfate broth;

d) Incubation at (44 ± 1) °C for (40 ± 4) h;

e) Detection of gas production, fluorescence and indole formation;

f) Quantification by the MPN technique.

6 Reagents, diluents and culture media

6.1 General instructions

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 demineralised or distilled water free from substances capable of inhibiting growth under the test conditions [ISO 8199:2005].

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

6.2 NaCl solution (0.9 % w/V)

Sodium chloride (NaCl)	9.0 g
Demineralised water	1000 mL

Mix the sodium chloride in water in a 2 000 mL flat bottom flask. Fill in 180 mL portions into 500 mL flasks (7.1) or in 9 mL portions into culture tubes (7.9). Adjust the pH to (7.0 ± 0.2) at 25°C.

Sterilize by autoclaving (7.3) at (121 ± 3)°C for (15 ± 2) min.

6.3 Fluorocult™, Lauryl sulfate broth with MUG

Trypsin digested peptone from meat	20.0 g
Lactose	5.0 g
Sodium chloride	5.0 g
Sodium lauryl sulphate	0.1 g
Di-potassium hydrogen phosphate	2.75 g
Potassium di-hydrogen phosphate	2.75 g
L- tryptophan	1.0 g
4-methyl-umbelliferyl- ß-D-glucuronide (MUG)	0,1 g
Demineralised water	1000 mL

Mix carefully the following substances in 1 000 mL of water in a 2 000 mL flat bottom flask, while heating in a boiling water bath (7.16). Adjust the pH (7.11) to (6.8 ± 0.1) using 1 mol/L sodium hydroxide solution. Distribute the solution in 9 mL portions into culture tubes and add a Durham tube (7.11) to each portion.

Sterilize in an autoclave (steam sterilizer) (7.3) for (15 ± 1) min at (121 ± 3)°C.

6.4 Kovacs reagent

4-di-methylamino benzaldehyde ($C_9H_{11}NO$)	5.0 g
Isoamyl alcohol ($C_5H_{12}O$)	75.0 mL
Hydrochloric acid (p = 1,18 g/mL)	25.0 mL

Dissolve the 4-dimethylamino benzaldehyde in the isoamyl alcohol and heat in a water bath at 60°C for 5 min. Then, add slowly the hydrochloric acid.

The reagent will be ready for use after 6 to 7 h (indicated by a yellow colour). Store in the refrigerator and protect from light.

WARNING - Kovacs reagent is harmful if swallowed, irritating to the respiratory system and to the skin. It is recommended to use it in a flow cabinet.

7 Apparatus

With the exception of equipment supplied sterile, the glassware shall be sterilised in accordance with the instructions given in ISO 8199: 2005.

Usual microbiological laboratory equipment, and in particular:

7.1 Wide-mouth glass flasks or beakers for example 125 mL, 200 mL, 500 mL and 2 000 mL

7.2 Thermostatic incubators regulated at (44 ± 1) °C (static)

7.3 Autoclave (Steam sterilizer)

7.4 Refrigerator

7.5 Sterile plastics Petri dishes, with lid of about 90 mm in diameter

7.6 Graduated pipettes, of nominal capacities 1 and 10 mL

7.7 Apparatus for shaking the culture tubes

7.8 Culture tubes, 25 mL capacity, or equivalent containers

7.9 Vortex mixer suitable for of 25 mL capacity culture tubes or equivalent containers

7.10 Durham-tubes.

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

7.12 Boiling water bath

7.13 UV-lamp (366 nm)

7.14 Laboratory spatula

7.15 Analytical balance

8 Sampling and hazards

Take samples of at least 100 g wet weight and deliver them to the laboratory as quickly as possible (within 24 h). In order to prevent propagation or inactivation of *E. coli* during transport to the laboratory and subsequent storage, refrigerate the sample at (5 ± 3) °C.

8.1 General

Samples are liable to ferment and can 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 bio-hazardous samples are followed.

See also the Warning note in Introduction.

8.2 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 no more than 72 h after receipt.

8.3 Handling

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

See also the Warning note in Introduction.

9 Procedure

9.1 Sample preparation

Place 20 g (wet weight) of sample into 180 mL sterile 0.9 % NaCl solution. Shake at a minimum of 150 rpm for 20 h at (5 ± 3)°C.

NOTE 1 **A 20 h shaking is recommended for all samples. In the case of liquid homogeneous samples (e.g. anaerobic digested sewage sludge) the shaking time may be reduced, but not less than 30 min.**

NOTE 2 **For disinfectant (e.g. lime, peracetic acid) treated sludges, a suitable pre-treatment for neutralising the disinfecting agent is required. For lime treated materials adjust the pH to (7,0 ± 0,5) with 1 mol/L hydrochloric acid. For other relevant chemicals (e.g. peracetic acid), a suitable neutralisation procedure must be used (see for example EN 1040 for neutralizers)**.

9.2 Analysis

Take an aliquot of 1 mL out of the primary prepared suspension (9.1) from sample preparation.

Prepare a serial tenfold dilution up to 10^{-7}: 1 mL of prepared suspension (9.1) + 9 mL of sterile 0.9 % NaCl solution.

From each dilution step, transfer 1 mL per tube (7.1) into 3 tubes containing 9 mL of Fluorocult™ lauryl sulfate broth with MUG and a Durham tube (6.2) each.

Incubate at (44 ± 1)°C for (40 ± 4) h.

Observe the Durham tube for gas formation. Consider all tubes with gas as positive culture.

Add 0.5 mL of NaOH 1N (6.4) to each “gas positive” tube and examine for fluorescence with 366 nm UV-light (7.17). Consider all fluorescent tubes as positive culture.

Add a 0,5 cm layer of Kovács reagent to each “gas and fluorescence positive” tube and watch for the colour change (cherry red after 1-2 min). Consider all tubes with a red circle as positive culture.

NOTE 1 **The number of dilution steps depends on type of matrix being tested. For untreated matrix a dilution up to 10^{-7} should be carried out. However, for treated matrix, dilution up to 10^{-4} should be sufficient.**

NOTE 2 **The primary solution (described in 9.1) is already diluted as 10^{-1}. From this primary dilution, transfer 1 mL into three tubes each containing 9 mL Fluorocult™ lauryl sulfate broth with MUG (6.2), as a first dilution step.**

NOTE 3 **if all tubes in the last three dilution steps (10^{-5}, 10^{-6} and 10^{-7} or 10^{-2}, 10^{-3} and 10^{-4}) are positive (gas+/fluorescence+/indole+), prepare 10^{-8} and 10^{-9} or 10^{-5} and 10^{-6} dilutions steps and transfer 1 mL per tube (5.1) into 3 tubes containing 9 mL Fluorocult™ lauryl sulfate broth with MUG (6.2) and a Durham tube (7.10) each. Store all tubes from the dilution steps of the sample at 4°C until the final result is obtained.**

9.3 Determination of the dry residue content

The numbers of *E. coli* may be calculated per wet weight or dry weight. For the latter, it is necessary to determine the dry residue of the sample using the method described in EN 12880. This shall be performed in parallel with the microbiological analysis.

10 Expression of the results

For each of the 7 dilutions (from 10^{-1} to 10^{-7}), note the number of positive tubes (gas+/fluorescence+/indol+) (between 0 and 3). Identify the characteristic number composed of 3 digits, corresponding to the number of positive tubes of the 3 last dilutions giving a number of positive tubes >0.

Calculate the MPN corresponding to the identified characteristic number using the De Man table (1983) [3], by multiplying the MPN index by the dilution factor. The result corresponds to a MPN per mL of primary prepared suspension.

For example:

	Gas+/Fluorescence+/Indol+						
Dilution step	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}+	10^{-7}+
Tube 1	+	+	+	+	-	-	-
Tube 2	+	+	+	-	+	-	-
Tube 3	+	+	+	-	-	-	-
Characteristic number	3	3	**3**	**1**	**1**	0	0
MPN index	7.5						
Dilution factor	10^{-3}						

Result: MPN *E.coli*/g (wet weight) sample	$7{,}5 \times 10^3$

The result per gram of sample material is MPN *E. coli*/g (wet weight) of original sample.

Numbers present per dry weight of sludge are calculated according to:

$$N_d = N_w \times 100/e$$

Where

Nd = bacterial count in MPN g dry weight

e = % dry mass of the original wet (as received) sample

and

N_w = bacterial count in MPN *E. coli*/g wet weight

11 Performance data

Performance data in terms of repeatability and reproducibility of the procedure, obtained by interlaboratory tests of the validation study (European scale Interlaboratory trial) performed during the FP6 EU Horizontal-Hyg project is given in Annex B (informative).

12 Test report

The test report shall contain the following information:

a) reference to this European standard;

b) all information necessary for complete identification of the sample;

c) details of sample pre-treatment, if carried out; and

d) results of the determination according to clause 9.

Any detail not specified in this European Standard and any other factor which may have affected the results.

Annex A
(informative)

MPN Statistical Table for 3-Tubes MPN procedure (de Man *et al.*, 1983)

Characteristic number			MPN index	Confidence limits			
1st digit	2nd digit	3rd digit		≥ 95 %	≥ 95 %	≥ 99 %	≥ 99 %
0	0	0	< 0.30	0.00	0.94	0.00	1.40
0	0	1	0.30	0.01	0.95	0.00	1.40
0	1	0	0.30	0.01	1.00	0.00	1.60
0	1	1	0.61	0.12	1.70	0.05	2.50
0	2	0	0.62	0.12	1.70	0.05	2.50
0	3	0	0.94	0.35	3.50	0.18	4.60
1	0	0	0.36	0.02	1.70	0.01	2.50
1	0	1	0.72	0.12	1.70	0.05	2.50
1	0	2	1.1	0.4	3.5	0.2	4.6
1	1	0	0.71	0.13	2.00	0.06	2.70
1	1	1	1.1	0.4	3.5	0.2	4.6
1	2	0	1.1	0.4	3.5	0.2	4.6
1	2	1	1.5	0.5	3.8	0.2	5.2
1	3	0	1.6	0.5	3.8	0.2	5.2
2	0	0	0.93	0.15	3.50	0.07	4.60
2	0	1	1.4	0.4	3.5	0.2	4.6
2	0	2	2.0	0.5	3.8	0.3	5.2
2	1	0	1.5	0.4	3.8	0.2	5.2
2	1	1	2.0	0.5	3.8	0.2	5.2
2	1	2	2.7	0.9	9.4	0.5	14.2
2	2	0	2.1	0.5	4.0	0.2	5.6
2	2	1	2.8	0.9	9.4	0.5	14.2
2	2	2	3.5	0.9	9.4	0.5	14.2
2	3	0	2.9	0.9	9.4	0.5	14.2
2	3	1	3.6	0.9	9.4	0.5	14.2
3	0	0	2.3	0.5	9.4	0.3	14.2
3	0	1	3.8	0.9	10.4	0.5	15.7
3	0	2	6.4	1.6	18.1	1.0	25.0
3	1	0	4.3	0.9	18.1	0.5	25.0
3	1	1	7.5	1.7	19.9	1.1	27.0
3	1	2	12	3	36	2	44
3	1	3	16	3	38	2	52
3	2	0	9.3	1.8	36.0	1.2	43.0
3	2	1	15	3	38	2	52
3	2	2	21	3	40	2	56
3	2	3	29	9	99	5	152
3	3	0	24	4	99	3	152
3	3	1	46	9	198	5	283
3	3	2	110	20	400	10	570
3	3	3	> 110				

Annex B
(informative)

Performance data of the interlaboratory comparison

B.1 Objective of the interlaboratory comparison

In a European wide laboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “Detection and enumeration of *Escherichia coli* in sludges, soils and treated biowaste. Part 3: Macromethod (MPN) in liquid medium” were established.

B.2 Material used in the interlaboratory comparison study

The interlaboratory comparison of the macromethod for quantification of *E. coli* in soil, sludge and treated biowaste took place from May to July 2007. It was carried out with 14 European laboratories on 7 different matrices. The matrices selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials (detailed information can be found in the final report on the interlaboratory comparison study [4]).

Table B.2-1 provides a list of the type of matrices chosen for *E. coli* detection.

Table B.2-1 Matrices types tested in the interlaboratory comparison trial

Mesophilic anaerobic digested sewage sludge
Anaerobic treated biowaste
Pelletised air-dried sludge
Digested sewage sludge presscake
Composted sewage sludge
Composted green waste
Composted biowaste

In the interlaboratory comparison study the following starting points were used:

The laboratory samples were all taken from a large batch of the different matrices according to the normal practice. The choice was made to analysed only spiked samples so as to obtain positive results. The spiking, the mixing and the sub-sampling were carried out as needed to prepare representative laboratory samples of approximately 150 g from the large batch sample. These were sent out by courier to each of the participating laboratories.

The experimental plan designed by project Horizontal-Hygiene on the basis of each laboratory being given 3 laboratory samples of each of the 2 batches of the seven matrices to be tested.

B.3 First assessment of the precision of the method

The statistical evaluation was conducted according to ISO 13843. The limit of detection, the upper limit of quantification, the range of quantification and the results of dispersion U^2 were obtained (Table B.3-1).

The limit of detection corresponds to the number of particles (germs per test portion) when the probability of a negative result is 5% (superior limit of the confidence interval of the null result).

Poisson distribution corresponds to the random distribution of the number of particles at the moment of sampling a perfectly homogenised suspension.

The relative variance U^2 corresponds to the relative standard deviation squared ratio of the standard deviation squared and the mean squared as:

$$U^2 = s^2/m^2$$

NOTE this statistic is commonly used to express dispersion or uncertainty of microbiological test results.

Table B.3-1 — Summary of components of the *E. coli* Macromethod precision

Limit of detection (5%) *E. coli* /g wet weight	**Upper limit of quantification (5%)** *E. coli* /g wet weight	**Range of quantification** Log10 unit	**Results of dispersion U^2**
8.99	$4.65\ 10^4$	5.7	0.8

NOTE In judging the results it is important to consider that they do not depend on the experimental data but only on the design of the measurement protocol (random variation).

B.4 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability (r) and the reproducibility (R) were obtained (Table B.4-1).

The repeatability corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another, both test results being obtained under the following conditions: the tests are performed in accordance with all the requirements of the present standard by the

same laboratory using its own facilities and testing laboratories samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship: $r_{test} = f.\sqrt{2}.s_{r.test}$ with the critical range factor f = 2.

> **NOTE** The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f.\sqrt{2}$ corresponds to the theoretical factor of 1.96 for a pure normal distribution at 95% statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). However, it may be necessary to a use a larger value for f in situations as described clause 12.

The reproducibility, like repeatability corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another test result obtained by another laboratory, both test results being obtained under the following conditions: the tests are performed in accordance with all the requirements of the present standard by two different laboratories using their own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

This reproducibility limit was calculated using the relationship: $R = f.\sqrt{2}.s_R$ with the critical range factor f = 2.

> **NOTE** The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f.\sqrt{2}$ corresponds to the theoretical factor of 1.96 for a pure normal distribution at 95% statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k.s_R$ with the usual value k = 2, resulting in a dispersion limit lower than the reproducibility limit (i.e. a ration of $\sqrt{2}$). However it may be necessary to use a larger value $f.\sqrt{2}$ (or k) in situation as described in clause 9.

In case of relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Table B.4-1 (this means that the value chosen for the critical range factor f is larger than 2 as well as for the coverage factor k for dispersion). This is because the extreme results may have been obtained in accordance with the present standard and/or be caused by the variability within, or in between, the laboratory samples.

For the calculations, as the test results were expressed on a log scale, the standard deviations in repeatability and Reproducibility conditions, respectively s_r and s_R were also expressed on a log scale. The expression of repeatability and Reproducibility in terms of maximum difference that can be expected between one test and another is given then by the limit of repeatability and Reproducibility respectively $r = 2\sqrt{2}.s_r$ and $R = 2\sqrt{2}.s_R$.

In order to make easier the interpretation, the values of repeatability and reproducibility are expressed in terms of maximal difference between two independent measurements on log scale, with a confidence level of 95%:

Example

Assuming r_1 and r_2 two independent measurements observed for a given method in repeatability conditions with $r_1 > r_2$:

$\log(r_1) - \log(r_2) \leq 0.9$ (95% of the cases), corresponding to almost 1 log of difference between results

The deviations between test results obtained under repeatability and reproducibility conditions can also be expressed by the maximal ratio between two independent measurements on natural scale (number of germs), with a confidence level of 95%

Example

$\log(r_1) - \log(r_2) \leq 0.9$ (95% of the cases)

then $r_1/r_2 \leq 10^{0.9}$

then $r_1/r_2 \leq 7.9$

thus r_1 is significantly higher than r_2 if $r_1/r_2 > 7.9$.

Table A.1 — Summary of *E. coli* Macromethod results of inter-laboratory comparison

Matrix	**Overall mean** (*E. coli* /g wet weight)	**Repeatability** (ratio)	**Reproducibility** (ratio)	**Discarded outliers** (statistical fitness)	**Removed data** (other reasons)	**Total number of data**	**Total number of labs**
Mesophilic anaerobic digested sewage sludge	< 8.99[(1)]	-	-	-	-	3	14
	< 8.99[(1)]	-	-	-	-	3	14
Anaerobic treated biowaste	832837	7.0	25.7	2	-	27	13
	1547828	10.8	61.9	-	-	33	13
Pelletised air dried sludge	< 8.99[(1)]	-	-	-	-	0	13
	394368	9.2	2632.7	-	-	27	14
Digested sewage sludge presscake	3898	9.7	73661.6	-	-	39	13
	1288	20.1	3505.1	-	-	39	13
Composted sewage sludge	> 4.65 10^{6}[(2)]	-	-	-	-	0	13
	833659	18.8	85.3	-	-	30	13
Composted green waste	818474	13.4	90.5	-	-	33	13
	193497	8.5	49.6	-	-	36	13
Composted biowaste	1531[(3)]	3255.0[(3)]	3255.0[(3)]	-	-	30	13
	> 4.65 10^{6}[(2)]	-	-	-	-	6	13

[(1)] Theoretical limit of detection with a probability of 95% calculated for the method

[(2)] Theoretical upper limit of quantification with a probability of 95% calculated for the method

[(3)] The observed variance was only random variation (no significant laboratory bias). Estimation to be considered carefully

NOTE In judging the results it is important to consider the concentrations levels, at which measurements have been carried out.

Bibliography

[1] CEN/TC 308 – doc 525:2001, Revision of Directive 86/278/EEC (3rd Draft).

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