

STN	Kvalita pôdy Odhad početnosti vybraných mikrobiálnych génových sekvencií kvantitatívnou PCR z DNA extrahovanej priamo z pôdy (ISO 17601: 2016)	STN EN ISO 17601 46 5273
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Soil quality - Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil (ISO 17601:2016)

Táto norma obsahuje anglickú verziu európskej normy.
This standard includes the English version of the European Standard.

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EUROPEAN STANDARD
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EN ISO 17601

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English Version

**Soil quality - Estimation of abundance of selected
microbial gene sequences by quantitative PCR from DNA
directly extracted from soil (ISO 17601:2016)**

Qualité du sol - Estimation de l'abondance de
séquences de gènes microbiens par amplification par
réaction de polymérisation en chaîne (PCR)
quantitative à partir d'ADN directement extrait du sol
(ISO 17601:2016)

Bodenbeschaffenheit - Abschätzung der Häufigkeit
ausgewählter mikrobieller Gensequenzen durch
quantitative PCR aus DNA-Boden-Extrakten (ISO
17601:2016)

This European Standard was approved by CEN on 14 February 2018.

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EN ISO 17601:2018 (E)**Contents**

Page

European foreword.....	3
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European foreword

The text of ISO 17601:2016 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 17601:2018 by Technical Committee CEN/TC 444 “Test methods for environmental characterization of solid matrices” the secretariat of which is held by NEN.

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Endorsement notice

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INTERNATIONAL STANDARD

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Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil

*Qualité du sol — Estimation de l'abondance de séquences de gènes
microbiens par amplification par réaction de polymérisation en
chaîne (PCR) quantitative à partir d'ADN directement extrait du sol*



Reference number
ISO 17601:2016(E)

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Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Test materials	4
5.1 DNA	4
5.2 Bacteria	4
5.3 Plasmid	4
5.4 Enzyme	4
5.5 Chemicals	4
5.6 Product for bacterial culture medium	5
5.7 Buffer and reagents	5
6 Apparatus	6
7 Procedure	6
7.1 qPCR standard preparation and calibration of qPCR assay (task 1)	6
7.1.1 General	6
7.1.2 Amplicon design (task 1, step 1)	6
7.1.3 qPCR standard preparation (task 1, step 2)	7
7.1.4 Isolate DNA, environmental DNA, artificial DNA	7
7.1.5 Calibration of the qPCR (task 1, step 3)	9
7.2 Preparation of soil DNA template and inhibition test (task 2)	10
7.2.1 General	10
7.2.2 Soil DNA preparation (task 2, step 4)	10
7.2.3 Inhibition test (task 2, step 5)	10
7.2.4 Dilution of DNA template	12
7.3 qPCR assay (task 3)	12
7.3.1 General	12
7.3.2 qPCR	12
7.4 Validation and analysis of qPCR assay (task 4)	12
7.4.1 General	12
7.4.2 Validation of the qPCR assay	12
7.4.3 Calculation of the copy number of the gene of interest in the soil DNA extract	13
8 Examination of the critical steps of the qPCR assay	14
9 Expression of the results of the qPCR assay	14
10 International ring test	14
11 Test report	14
Annex A (informative) Description of principal steps of TaqMan® qPCR assay	15
Annex B (informative) International ring-test for evaluating qPCR to quantify the abundance of selected microbial gene sequences from DNA directly extracted from soil	17
Bibliography	30

ISO 17601:2016(E)**Foreword**

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

DNA (DNAs) is a major component of any living organisms coding for enzymes responsible for their biological activities. The study of DNA sequences from DNA sources extracted from different environmental matrices, by means of numerous molecular approaches, provides molecular markers that can be used to sharply distinguish and identify different organisms (bacteria, *archaea*, and *eucaryotes*).

Up to now, most of the studies aiming to develop microbial quality indicators applicable to complex environment such as soil were biased by the poor culturability of many microorganisms under laboratory conditions and the lack of sensitivity of traditional microbiological methods. The recent development of a large set of molecular biology methods based on amplification of soil-extracted nucleic acids have provided a pertinent alternative to classical culture-based microbiological methods providing unique insight into the composition, richness, and structure of microbial communities.^[2] ^[3] ^[4] ^[5] ^[6] DNA-based approaches are now well established in soil ecology and serve as genotypic markers for determining microbial diversity. The results of molecular analyzes of soil microbial communities and/or populations rely on two main parameters: a) the extraction of DNA representative of the indigenous bacterial community composition and b) PCR bias such as the choice of primers, the concentration of amplified DNA, errors in the PCR, or even the method chosen for analysis.^[7] ^[4] ^[8] ^[9]

Numerous studies have investigated new methods to improve extraction, purification, amplification, and quantification of DNA from soils.^[10] Recently, ISO 11063 reporting “a method to extract nucleic acids directly from soil samples” derived from Reference ^[10] is opening a new window for developing standardized molecular approaches to estimate soil quality.^[11]

The aim of this International Standard is to describe the procedure used to set up and perform quantitative PCR to quantify the abundance of soil microbial phyla, as well as functional groups from DNA directly extracted from soil samples. The quantification of soil microbial phyla, as well as functional groups by qPCR assays can contribute to the development of routine tools to monitor soil quality. The repeatability and the reproducibility of the procedure of the quantitative PCR were assessed in an international ring test study (see [Annex B](#)). The repeatability of this procedure was successfully evaluated for both 16S rRNA genes, as well as genes coding a functional marker of denitrifiers (the nitrite reductase gene *nirK*). The reproducibility of this procedure revealed a laboratory effect which can be overcome by interpreting the results of the quantification of the abundance of the microbial groups by comparison, either by using an external reference (DNA extracted from a control strain) in the assay or by calculating a percentage of variations between treatments to normalize the data.

Soil quality — Estimation of abundance of selected microbial gene sequences by quantitative PCR from DNA directly extracted from soil

1 Scope

This International Standard specifies the crucial steps of a quantitative real-time polymerase chain reaction (qPCR) method to measure the abundance of selected microbial gene sequences from soil DNA extract which provides an estimation of selected microbial groups.

It is noteworthy that the number of genes is not necessarily directly linked to the number of organisms that are measured. For example, the number of ribosomal operon is ranging from one copy to 20 copies in different bacterial phyla. Therefore, the number of 16S rRNA sequences quantified from soil DNA extracts does not give an exact estimate of the number of soil bacteria. Furthermore, the number of sequences is not necessarily linked to living microorganisms and can comprise sequences amplified from dead microorganisms.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 11063, *Soil quality — Method to directly extract DNA from soil samples*

koniec náhľadu – text ďalej pokračuje v platenej verzii STN