

STN	Kvalita pôdy Identifikácia ekotoxikologických skúšobných druhov DNA čiarovým kódovaním (ISO 21286: 2019)	STN EN ISO 21286 46 5262
------------	---	--

Soil quality - Identification of ecotoxicological test species by DNA barcoding (ISO 21286:2019)

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

Táto norma bola oznámená vo Vestníku ÚNMS SR č. 08/20

Obsahuje: EN ISO 21286:2020, ISO 21286:2019

EUROPEAN STANDARD

EN ISO 21286

NORME EUROPÉENNE

EUROPÄISCHE NORM

April 2020

ICS 13.080.30

English Version

Soil quality - Identification of ecotoxicological test species by DNA barcoding (ISO 21286:2019)

Qualité du sol - Identification des espèces par codes-
barres ADN dans les essais d'écotoxicologie (ISO
21286:2019)

Bodenbeschaffenheit - Allgemeine Anleitung zur
Verwendung des DNA-Barcodes in ökotoxikologischen
Untersuchungen (ISO 21286:2019)

This European Standard was approved by CEN on 13 April 2020.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

EN ISO 21286:2020 (E)**Contents**

Page

European foreword.....	3
-------------------------------	----------

European foreword

The text of ISO 21286:2019 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 21286:2020 by Technical Committee CEN/TC 444 "Environmental characterization of solid matrices" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2020, and conflicting national standards shall be withdrawn at the latest by October 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 21286:2019 has been approved by CEN as EN ISO 21286:2020 without any modification.

INTERNATIONAL STANDARD

ISO
21286

First edition
2019-03

Soil quality — Identification of ecotoxicological test species by DNA barcoding

*Qualité du sol — Identification des espèces par code-bare ADN dans
les essais d'écotoxicologie*



Reference number
ISO 21286:2019(E)

© ISO 2019

ISO 21286:2019(E)**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Reagents and material	3
5.1 Biological material	3
5.2 Enzyme	3
5.3 Oligonucleotide PCR primers	3
5.4 Reagents	3
6 Apparatus	4
7 General requirements	5
7.1 Experimental precaution and contamination avoidance	5
7.2 Safety precautions	5
7.2.1 Chemical hazards	5
7.2.2 Physical hazards	6
8 Procedure	6
8.1 DNA isolation	6
8.2 Quantification	7
8.3 PCR	7
8.3.1 Target genomic region	7
8.3.2 Primer design	7
8.3.3 Primer synthesis	7
8.3.4 PCR	8
8.4 Checking the amplicon size	9
8.5 Purification	9
8.6 Sequencing	9
8.7 Bioinformatics	9
8.7.1 General	9
8.7.2 Electropherogram or raw sequence quality checking	10
8.7.3 Trimming of low-quality regions and primers sequences	10
8.7.4 Sequence overlapping	10
8.7.5 Sequence verification	11
8.7.6 Reviewing the edited sequence	11
8.7.7 Species assignment	11
8.7.8 Quality of the reference databases	12
9 Calculation and expression of results	13
10 Validity of the test	13
11 Test report	14
Annex A (informative) <i>Eisenia</i> Barcoding Initiative: A ring test to evaluate the applicability of DNA barcoding for the identification of <i>Eisenia</i> species	15
Bibliography	18

ISO 21286:2019(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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Currently, test species identification is usually based on morphological characters. However, this does not always give clear results because

- a) few taxonomic experts are available,
- b) closely related species can differ by a few, easily overlooked characters, and
- c) even more importantly, several test species are in fact complexes of cryptic species.

A good example is the compost worm *Eisenia fetida/andrei* (used in ISO 11268-1, ISO 11268-2 and ISO 17512-1), in which morphological traits alone may not be sufficient to discriminate between both species[5][36]. Another well-known case is the predatory mite, *Hypoaspis* (*Geolaelaps*) *aculeifer*[50], which might get confused with *H. miles*, widely used in biological pest control[31].

Species misidentifications, the use of a morphospecies which is actually a complex of cryptic species, or even species mixing in lab cultures, can be a serious problem for the reliability of the ecotoxicological tests. Sibling species in a morphospecies complex can exhibit ecological, behavioural, and physiological differences, and can differ also in their response to toxicants (e.g. References [2], [17], [35], [40]). This also seems to be the case of the springtail *Folsomia candida* (used in ISO 11267 and ISO 17512-2), in which considerable levels of genetic differentiation have been found among natural populations of *F. candida* and among laboratory strains[9][19][41]. Although different laboratory strains have been found to exhibit only minor differences in the sensitivity towards some chemicals[12][9], other studies have detected significant variation in phenmedipham avoidance behaviour and divergent fitness responses to cadmium exposure among genetically differentiated strains[14][30]. Moreover, even if two species have similar responses to toxicants, the presence of two species within the same laboratory culture can result in the production of sterile hybrids, which will bias the outcome of reproduction tests[36].

Implementing species identification via DNA barcoding can help to overcome these obstacles, ensuring that the species or strain used for testing is well characterized. As a result, quality assurance can be improved, making the results obtained by different ecotoxicological laboratories far more reliable and comparable. For *Eisenia fetida/E. andrei* this work, including an international ringtest, has already been performed[36], see [Annex A](#). The conclusions of this ringtest can be summarized as follows.

- DNA barcoding is a reliable and practical method for identifying *Eisenia* species.
- Only 17 out of 28 ecotoxicological laboratories were correct in their taxonomic assignment. Most laboratories with wrong or unknown assignments actually have *E. andrei* in stock.
- The existence of a cryptic species pair within *E. fetida* is a plausible hypothesis.
- It is important that earthworms used for ecotoxicological tests are regularly (re-)identified by DNA barcoding.

Very probably, similar experiences and recommendations can be drawn for other invertebrates species used in terrestrial ecotoxicology, as well as plants. Indeed, DNA barcoding has proven to be useful for specimen identification and species delimitation in many organism groups, including other earthworms[13][37], enchytraeids[16], mites[15], collembolans[32], molluscs[42], nematodes[28] and terrestrial plants[8].

Soil quality — Identification of ecotoxicological test species by DNA barcoding

1 Scope

This document specifies a protocol to identify ecotoxicological test specimens (mainly invertebrates and plants) to the species level, based on the DNA barcoding technique. This protocol can be used by laboratories performing DNA barcoding in order to standardize both the wet-lab and data analysis workflows as much as possible, and make them compliant with community standards and guidelines.

This document does not intend to specify one particular strain for each test method, but to accurately document the species/strain which was used.

NOTE 1 This does not imply that DNA barcoding is performed in parallel to each test run, but rather regularly (e.g. once a year, such as reference substance testing) and each time a new culture is started or new individuals are added to an ongoing culture.

This document does not aim at duplicating or replacing morphological-based species identifications. On the contrary, DNA barcoding is proposed as a complementary identification tool where morphology is inconclusive, or to diagnose cryptic species, in order to ensure that the results obtained from different ecotoxicological laboratories are referring to the same species or strain.

This document is applicable to identifications of immature forms which lack morphological diagnostic characters (eggs, larvae, juveniles), as well as the streamline identification of specimens collected in field monitoring studies, where large numbers of organisms from diverse taxa are classified.

NOTE 2 In principle, all species regularly used in ecotoxicological testing can be analysed by DNA barcoding. Besides the earthworms *Eisenia fetida* and *E. andrei*, further examples for terrestrial species are *Lumbricus terrestris*, *L. rubellus*, *Allolobophora chlorotica*, *Aporrectodea rosea*, and *A. caliginosa*, *Dendrodrilus rubidus*, *Enchytraeus albidus*, and *E. crypticus* (Haplotaxida); *Folsomia candida*, *F. fimetaria*, *Proisotoma minuta*, and *Sinella curviseta* (Collembola); *Hypoaspis aculeifer* and *Oppia nitens* (Acari); *Aleochara bilineata* and *Poecilus cupreus* (Coleoptera); *Scathophaga stercoraria*, *Musca autumnalis* (Diptera) or *Pardosa* sp. (Arachnida). Nematodes or snails and even plants can also be added to this list.

2 Normative references

There are no normative references in this document.

koniec náhľadu – text ďalej pokračuje v platenej verzii STN