INTERNATIONAL STANDARD

ISO 20395

First edition
2019-08

Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR

Biotechnologie — Exigences relatives à l'évaluation de la performance des méthodes de quantification des séquences d'acides nucléiques cibles — qPCR et dPCR

Reference number
ISO 20395:2019(E)

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

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 of 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*.

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

This document has been developed to specifically support the analytical requirements with respect to quantification of specific nucleic acid sequences (targets). It can also benefit the broader biomanufacturing, bioscience research and development, industrial biotechnology, engineering biology and advanced therapeutics industries which need to demonstrate product quality based on measurement and quantification of specific nucleic acid targets.

Quantification of nucleic acid target sequences is a cross-cutting fundamental measurement that broadly impacts many aspects of biotechnology. For example, quantification of nucleic acid biomarkers for monitoring bioprocess efficiency and conformity with quality by design parameters for biopharmaceutical manufacture and industrial biotechnology, characterization of purity and quality of cell-derived advanced therapy medicinal products (ATMPs); assessment of gene copy number for evaluating the potency and efficacy of gene-based therapies and process control assays for gene editing and engineering biology applications.

The underpinning technique of polymerase chain reaction (PCR) has transformed the field of nucleic acid analysis, due to its robustness and simplicity. Technological advances in instrumentation have resulted in a wide range of PCR-based nucleic acid quantification approaches/instruments with subsequent developments such as:

— quantitative real-time PCR (qPCR) which offers methods for quantification of DNA and RNA molecules relative to a calibration material or independent sample, and

— digital PCR (dPCR) which offers the ability to perform SI traceable quantification through the concept of molecular enumeration without the need for a calibration curve.

However, performing nucleic acid quantification assays to a high standard of analytical quality can be challenging. For example, it is well known that impure or degraded nucleic acid extracts can affect the accuracy of quantification. Similarly, a poorly designed qPCR or dPCR assay with poor amplification efficiency and primer specificity will have an impact on accuracy of quantification. In addition, aspects such as calibrators, standard curves, data normalization and processing can have a large influence on the accuracy of quantitative measurement of nucleic acid targets.

This document is expected to improve confidence in the data produced, support selection and optimization procedures and provide supporting performance parameters that may be utilized during performance qualification of a particular measurement procedure for quantification of nucleic acid target sequences. Biotechnology and bioscience industry data with higher measurement confidence will enable data interoperability, improved product quality, reduced risks and costs and facilitate international trade.

In this document, the following verbal forms are used:

— “shall” indicates a requirement;

— “should” indicates a recommendation;

— “may” indicates a permission;

— “can” indicates a possibility or a capability.

Further details can be found in the ISO/IEC Directives, Part 2.

Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR

1 Scope

This document provides generic requirements for evaluating the performance and ensuring the quality of methods used for the quantification of specific nucleic acid sequences (targets).

This document is applicable to the quantification of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) target sequences using either digital (dPCR) or quantitative real-time PCR (qPCR) amplification technologies. It applies to target sequences present in nucleic acid molecules including double-stranded DNA (dsDNA) such as genomic DNA (gDNA) and plasmid DNA, single stranded DNA (ssDNA), complementary DNA (cDNA), and single stranded RNA (ssRNA) including ribosomal RNA (rRNA), messenger RNA (mRNA), and long and short non-coding RNA [microRNAs (miRNAs) and short interfering RNAs (siRNAs)], as well as double-stranded RNA (dsRNA).

This document applies to nucleic acids derived from biological sources such as viruses, prokaryotic and eukaryotic cells, cell-free biological fluids (e.g. plasma or cell media) or in vitro sources [e.g. oligonucleotides, synthetic gene constructs and in vitro transcribed (IVT) RNA].

This document is not applicable to quantification of very short DNA oligonucleotides (<50 bases).

This document covers:

— analytical design including quantification strategies (nucleic acid copy number quantification using a calibration curve as in qPCR or through molecular counting as in dPCR, quantification relative to an independent sample and ratio measurements) and use of controls;

— quantification of total nucleic acid mass concentration and quality control of a nucleic acid sample including assessment of nucleic acid quality (purity and integrity);

— PCR assay design, optimization, in silico and in vitro specificity testing;

— data quality control and analysis including acceptance criteria, threshold setting and normalization;

— method validation (precision, linearity, limit of quantification, limit of detection, trueness and robustness) with specific requirements for qPCR and dPCR;

— approaches to establishing metrological traceability and estimating measurement uncertainty.

This document does not provide requirements or acceptance criteria for the sampling of biological materials or processing of biological samples (i.e. collection, preservation, transportation, storage, treatment and nucleic acid extraction). Nor does it provide requirements and acceptance criteria for specific applications (e.g. food or clinical applications where specific matrix issues can arise).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO/IEC Guide 98-3:2008, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)*

ISO/IEC Guide 99, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 99 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
amplicon
specific DNA fragment produced by a DNA-amplification technology, such as the polymerase chain reaction (PCR)

[SOURCE: ISO 13495:2013, 3.3.1]

3.2
amplification plot
graph representing the generation of a reporter (usually fluorescent) signal during a qPCR or dPCR reaction

Note 1 to entry: For qPCR and some dPCR systems, the amplification plot shows the relationship between cycle number (x-axis) and fluorescence signal (y-axis).

Note 2 to entry: For end point dPCR, the fluorescent signal of each dPCR partition is displayed. For a single fluorophore, a one-dimensional amplification plot shows partition number (x-axis) against end point fluorescent signal (y-axis). A multi-dimensional amplification plot shows fluorescent signal for each detector channel on each axis.

3.3
calibration curve
standard curve
expression of the relation between indication and corresponding measured quantity value

[SOURCE: ISO/IEC Guide 99:2007, 4.31, modified — The notes have been deleted.]

3.4
calibrator
measurement standard used in calibration

Note 1 to entry: The term “calibrator” is only used in certain fields.

EXAMPLE A qPCR interplate calibrator sample is often included on each qPCR plate in a study comprising multiple qPCR plates or experiments to compensate for variations across plates due to instrument measurement factors such as baseline and threshold setting. The interplate calibrator contains the target sequence(s) detected by the PCR assay and is measured with the same PCR assays as the studied samples.

[SOURCE: ISO/IEC Guide 99:2007, 5.12, modified — The example has been added.]

3.5
cDNA
complementary DNA
single-stranded DNA, complementary to a given RNA and synthesised in the presence of reverse transcriptase to serve as a template for DNA amplification