Technical Specification

ISO/TS 21085

Biotechnology — General requirements for the measurement of ultra-low concentration samples of target nucleic acid sequence

Biotechnologie — Exigences générales relatives au mesurage des échantillons à très faible concentration de séquence d'acide nucléide cible

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Foreword

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This document was prepared by Technical Committee ISO/TC 276, *Biotechnology*, Subcommittee SC 1, *Analytical Methods*.

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

When measuring ultra-low concentration of nucleic acid samples, the measurements often exhibit significant variation. This large variation is primarily caused by the Poisson distribution and other contributing factors. In addition, the ultra-low concentration makes the samples highly susceptible to issues such as adsorption and contamination. This document summarizes the key considerations and requirements for handling, measurement including both qualification and quantification analysis, and storage of ultra-low concentration sample of target nucleic acids sequence.

Analytical methods based on polymerase chain reaction (PCR) are theoretically capable of detecting the presence of one molecule of nucleic acid in the reaction solution, making it possible to amplify and measure the ultra-low concentration target nucleic acid sequence. These methods are used not only in the field of biotechnology, but also in many other areas such as medicine, food, and the environment. However, it is difficult to stably handle, measure, and store ultra-low concentration target nucleic acid sequences. For example, nucleic acids readily adsorb to polypropylene (PP) materials, which is widely used as a base material for labware, so target nucleic acid sequences stored in such containers are easily removed from the sample/reaction solution during storage or experiment and become undetectable. Similarly, if a sample before PCR and a sample after PCR are handled in the same place for the same target nucleic acid sequence, contamination can occur, and the measurement result will change. Furthermore, when the target nucleic acid sequence is present at ultra-low concentrations, the distribution of nucleic acid molecules becomes non-uniform. These phenomena can occur even in samples of general concentrations, but the effects of adsorption and contamination can be relatively small and negligible at higher concentrations. However, since measurement results on ultra-low concentration samples can be impacted significantly, there are special considerations to be taken into account when handing with such samples.

This document provides specific requirements to improve data reliability and obtain accurate measurement results when working with ultra-low concentrations of target nucleic acid sequence. Biotechnology and bioscience industry data with higher measurement confidence will enable data interoperability, improved product quality, reduced risks and costs and facilitate international trade.

Biotechnology — General requirements for the measurement of ultra-low concentration samples of target nucleic acid sequence

1 Scope

This document specifies requirements and considerations for handling, measuring, and storing samples with ultra-low concentration of target nucleic acid sequences, i.e., concentrations corresponding to copy numbers.

This document is applicable to nucleic acid detection methods (qPCR, dPCR, isothermal amplification, and NGS).

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 20395, *Biotechnology — Requirements for evaluating the performance of quantification methods for nucleic acid target sequences — qPCR and dPCR*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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 https://www.electropedia.org/

3.1
ultra-low concentration sample
<target nucleic acid sequence> sample containing 3 copies/µL to 100 copies/µL [10] of target nucleic acid sequences

Note 1 to entry: Ultra-low concentration sample of target nucleic acid sequence includes synthetized nucleic acid solution diluted with TE buffer.

3.2
copy number
number of molecules (copies) containing a specific nucleic acid sequence

[SOURCE: ISO 20395:2019, 3.6]

3.3
copy number concentration
number of molecules (copies) containing a specific nucleic acid sequence in a defined volume

[SOURCE: ISO 20395:2019, 3.7]

3.4
negative nucleic acid target control
NTC
reference nucleic acid, or nucleic acid extracted from a certified reference material, or known negative sample not containing the sequence under study

Note 1 to entry: This control demonstrates that the results of analyses of test samples not containing the target sequence will be negative.

[SOURCE: ISO 24276:2006, 3.4.2; modified — DNA replaced by nucleic acid.]

3.5
limit of detection
LOD
measured quantity value, obtained by a given measurement procedure, for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

[SOURCE: ISO 20395:2019, 3.14]

3.6
probability of detection
POD
proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration

Note 1 to entry: For qualitative methods, POD represents the probability of detection.

[SOURCE: ISO 16140-4:2020, 3.9]

3.7
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes to entry deleted.]

3.8
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.12, modified — Notes to entry deleted.]

3.9
precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — Notes to entry deleted.]

3.10
repeatability
measurement precision under a set of repeatability conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.21]

3.11
intermediate precision
measurement precision under a set of intermediate precision conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.23, modified — Note to entry deleted.]

3.12
reproducibility
measurement precision under reproducibility conditions of measurement

[SOURCE: ISO/IEC Guide 99:2007, 2.25, modified — Note to entry deleted.]

3.13
measurement uncertainty
non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used

[SOURCE: ISO/IEC Guide 99:2007, 2.26, modified — Notes to entry deleted.]

3.14
robustness
measure of a test method's capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage

[SOURCE: ISO 23033:2021 3.44]

3.15
test sample
sample prepared for testing or analysis, the whole quantity or part of it being used for testing or analysis at one time

[SOURCE: ISO 16577:2016, 3.210]

4 Principle

In this document, the following two patterns are assumed for the ultra-low concentration sample of target nucleic acid sequence.

a) Samples with ultra-low concentrations of both the matrix-derived nucleic acids and the target nucleic acid sequence.

   EXAMPLE 1 Environmental DNA, NA derived from highly processed food, samples in forensic science, fossil, series of standard for the validation and the verification without carrier NA.

b) Samples with an ultra-low concentration of the target of nucleic acid sequence and a high concentration of matrix-derived nucleic acids.

   EXAMPLE 2 GMO, samples in medical laboratory, microbiology.

When considering the sample specified in 4 a), the effects of the adsorption can be significant, while the effect of the adsorption can be negligible for the sample specified in 4 b). Variation due to Poisson distribution and other factors can affect both samples.

5 Requirements

5.1 General

There are several points to consider for the measurement of ultra-low concentration samples of target nucleic acid sequence compared to the measurement for those of general concentrations. In addition, since the impact of contamination is relatively greater in ultra-low concentration nucleic acid measurements than in general-concentration nucleic acid measurements, efforts to prevent contamination shall be implemented according to the purpose of the measurement. Examples of specific points to consider are described in the relevant subclauses.