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Biotechnology — Minimum requirements for optical signal measurements in photometric methods for biological samples

Biotechnologie — Exigences minimales relatives aux mesures de signaux optiques dans les méthodes photométriques pour les échantillons biologiques

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

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Foreword

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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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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 defines terms and provides general guidance for accurate measurement of optical signals used for analysis of biological samples in photometric methods. These photometric methods can use optical signal measurements, including bioluminescence, chemiluminescence, fluorescence or absorption measurement, that can be applied in the fields of biotechnology, life science and medicine. A measured optical signal value is applied for evaluating biological parameters qualitatively or quantitatively, including cellular and metabolic activities, and gene expressions. Photometric methods are used in applications such as toxicity testing, environmental risk assessment, biomanufacturing, drug development, regenerative medicine and biobanking.

There are significant needs for both manufacturers and users for high quality optical signal measurement in photometric methods in industry to increase confidence in the repeatability, intermediate precision and reproducibility for analysis of biological samples. While repeatability of the photometric method is already sufficient for qualitative characterization of biological samples, quantitative characterization requires more accurate intermediate precision and reproducibility of optical signal measurement. It requires proper optical signal measurements, and it also requires assessment of deviations from the ideal proportionality of the optical signal and the output of the photometric method. Requirements for proper optical signal measurement are an important component of the description of specific applications of photometric methods.

This document provides a general framework to support proper measurement of an optical signal in a photometric method. It focuses on the utilization of optical references and relevant technical issues for optical signal measurement in photometric methods, including procedures for verification of instruments, continual performance monitoring of instruments and photometric method validation. Optical references can be used to verify instruments to increase confidence in the repeatability, intermediate precision, and reproducibility of optical signal measurement. For example, an optical signal emitted from biological samples can be compared on a common measurement scale within a laboratory, between manufacturer and manufacturer, manufacturer and user, or user and user.

Biotechnology — Minimum requirements for optical signal measurements in photometric methods for biological samples

1 Scope

This document specifies minimum requirements to support accurate measurement of optical signals in photometric methods used for qualitative or quantitative characterization of biological samples.

This document is applicable to optical signals that are generated, for example, by bioluminescence, chemiluminescence and fluorescence, and optical signals that are detected as changes of light due to absorption.

This document addresses the verification of optical signal measurement instruments used in photometric methods for measurement of biological samples including considerations for the use of optical references.

This document does not provide sector- or application-specific performance criteria for the workflow of measuring biological samples. When applicable, users can also consult existing sector- or application-specific standards, or both.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology 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
accuracy
closeness of agreement between a measured quantity value and a true quantity value of a measurand

Note 1 to entry: The concept “measurement accuracy” is not a quantity and is not given a numerical quantity value. A measurement is said to be more accurate when it offers a smaller measurement error.

Note 2 to entry: The term “measurement accuracy” should not be used for measurement trueness and the term “measurement precision” should not be used for “measurement accuracy”, which, however, is related to both these concepts.

Note 3 to entry: “Measurement accuracy” is sometimes understood as closeness of agreement between measured quantity values that are being attributed to the measurand.

Note 4 to entry: ISO 5725-1:1994 uses a different definition for “accuracy”.

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — “measurement accuracy” and “accuracy of measurement” deleted as terms. Note 4 to entry added.]

3.2
biological sample
material or object of biological origin

3.3
dynamic range
range of *optical signal* (3.6) values that can be measured quantitatively

[SOURCE: ISO 2041:2018, 3.4.17, modified — "optical signal" and "quantitatively" added to the definition.]

3.4
light source
optical device emitting appropriate wavelength(s) in a specified spectral region

Note 1 to entry: A light source can be a part of an *optical signal* (3.6) measurement instrument.

[SOURCE: ISO 25178-604:2013, 2.3.1 modified — "wavelength(s)" replaced "range of wavelengths". Note 1 to entry added.]

3.5
optical reference
material, *light source* (3.4) or photodetector, sufficiently reproducible and stable with respect to optical properties, that has been established to be fit for its intended use

EXAMPLE Light emitting diode (LED)-based *reference light source* (3.11), laser, slide of fluorescent glass, fluorescent dye in solution or other matrix (e.g. fluorescent bead), slide embedded fluorescent material, reference filter, reference cuvette, reference film, reference solution, *power meter* (3.9) (see Annex B).

Note 1 to entry: The term "optical reference" includes both uncalibrated references and calibrated standards. Optical references can be distributed by an internal organization or prepared by a laboratory (e.g. in-house standard, in-house reference material).

Note 2 to entry: Optical references can be used for *verification* (3.14) of *optical signal* (3.6) measurement instruments (see Annexes D, E, G, H, I and J).

3.6
optical signal
light emitted or changes of light due to absorption caused by transmitting light through samples or chromogenic substances

Note 1 to entry: The optical signal measurement involves, for example, bioluminescence, chemiluminescence, fluorescence and absorption measurements. Annex A gives information about optical signals.

Note 2 to entry: In this document, the term "optical signal" focuses on light before detection.

3.7
optical signal intensity
strength of an *optical signal* (3.6)

Note 1 to entry: Intensity can be used to express the absolute strength or relative strength of an optical signal. An appropriate unit can be used in order to express the intensity of a particular optical signal.

3.8
photometric method
analytical technique using *optical signal* (3.6) measurement(s) to determine components or biological parameters of *biological samples* (3.2)

Note 1 to entry: The photometric method includes preanalytical, optical signal measurement and data analysis procedures.

Note 2 to entry: Biological parameters of biological samples include, for example, cellular and metabolic activities, and gene expressions.

Note 3 to entry: Examples for representative photometric methods are shown in Annex B.

Note 4 to entry: Analysis and assay results of photometric methods can be expressed qualitatively or quantitatively.

Note 5 to entry: The term "radiometric" is widely used instead of "photometric" in the field of optical engineering (e.g. IEC 60050-845).

3.9
power meter
optical power meter
measurement device to determine the radiant power of light used as an *optical reference* (3.5)

Note 1 to entry: The watt (W) is used as a unit to express radiant power.

3.10
precision
closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions

Note 1 to entry: Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.

Note 2 to entry: The "specified conditions" can be, for example, repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement (see ISO 5725-3:1994).

Note 3 to entry: Measurement precision is used to define measurement repeatability, intermediate measurement precision, and measurement reproducibility.

Note 4 to entry: Sometimes "measurement precision" is erroneously used to mean measurement accuracy.

Note 5 to entry: ISO 5725-1:1994 uses a different definition for "precision".

[SOURCE: ISO/IEC Guide 99:2007, 2.15, modified — "measurement precision" deleted as a term. Note 5 to entry added.]

3.11
reference light source
light source (3.4) used as an *optical reference* (3.5)

EXAMPLE Characterized or calibrated LED and laser.

3.12
reference material for calibration curve
material with known value of concentration or amount of a specific substance, for intended purpose

Note 1 to entry: It is identical to or commutable with the measurement object of a *biological sample* (3.2).

Note 2 to entry: Examples for expressing concentration and amount are mol/l and mol, respectively.

3.13
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The word "validated" is used to designate the corresponding status.

Note 3 to entry: The use conditions for validation can be real or simulated.

Note 4 to entry: ISO/TS 16393:2019 uses the term “validation” in a different meaning in defining “validation experiment”. ISO/IEC Guide 99:2007 uses a different definition for “validation”.

[SOURCE: ISO 9000:2015, 3.8.13, modified — Note 4 to entry added.]

3.14
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Note 1 to entry: The objective evidence needed for a verification can be the result of an inspection or of other forms of determination such as performing alternative calculations or reviewing documents.

Note 2 to entry: The activities carried out for verification are sometimes called a “qualification process”.

Note 3 to entry: The word “verified” is used to designate the corresponding status.

Note 4 to entry: ISO/IEC Guide 99:2007 uses a different definition for “verification”.

[SOURCE: ISO 9000:2015, 3.8.12, modified — Note 4 to entry added.]

4 Principles

4.1 General

Optical signal measurements, including bioluminescence, chemiluminescence, fluorescence and absorption measurements, are used in photometric methods. Optical signal measurements are often used for biological samples to determine a diverse set of biological parameters qualitatively and quantitatively, including cellular and metabolic activities, and gene expressions (see Annex A for more information). In the photometric methods, the optical signal intensity and spectrum from biological samples are measured using instruments.

NOTE 1 Examples of instruments are luminometers, imaging analysers, fluorescence plate readers, flow cytometers, microarray readers, spectrofluorometers, plate readers, spectrophotometers and DNA sequencers (see Annex B).

Accuracy, precision, repeatability and reproducibility represent some of the important metrological factors used for evaluating the effectiveness of photometric method applied.

Photometric methods can be qualitatively validated using positive and negative control materials.

NOTE 2 The performance characteristics of qualitative photometric methods and their validation can be determined with appropriate statistical models depending on the method, structure of data and statistical experience (e.g. ISO/TS 16393).

Accurate analysis and assay results are obtained by measuring the optical signal with an appropriate selection of experimental materials, including the reagents generating the optical signal from the sample, and the use of suitable instruments for the intended purpose.

Sample preparation is also an important factor governing the performance of a photometric method.

Optical signal measurements produce relative and absolute optical signal values that are functionally related to the quantity of specific characteristics of biological samples or biological parameters. In spectral-resolved measurements, spectral characteristics are indicative for the interaction of particular molecules, structural elements of molecules, or molecular interaction with electromagnetic radiation of different energy.

In some cases, calibration curves constructed using a reference material for calibration curve are required for quantification of the absolute amount of biological sample. A calibration curve can be also

used to determine an effective amount of a test article (e.g. an amount that elicits 50 % response across the calibration curve or ED_{50}).

NOTE 3 Annex H gives an example for the construction of a calibration curve.

For measurement of biological samples, it is sometimes necessary to label or stain biological samples, introduce a reporter gene into cells, tissues and whole organisms, or trigger chemical reactions.

NOTE 4 Reagent quality and its photophysical and chemical properties affects optical signals from the sample. Activity of cells can sometimes affect optical signals.

NOTE 5 Ambient light radiation can cause deterioration of bioluminescent reagents, chemiluminescent reagents, fluorescent materials and fading absorption.

When cells are used in photometric methods, the robustness of analysis and assay results is less reliable if the cellular activity is unstable. In particular, optical signal measurement results are directly affected by the stability of the cellular activity during long-term storage/subculturing and by the stability of responsiveness to the target bioactive substance. The incident measuring light can also affect cellular functions and properties, in particular if the cells are exposed to the light for a long period. Accordingly, the reliability of optical signal measurement results can be increased by maintaining cell stability.

NOTE 6 Examples are assays to evaluate cellular activity, including viability, toxicity and metabolic activity by means of cell-based assays.

NOTE 7 Relevant standards that describe representative methods by means of optical signal measurements are listed in Annex C.

Preanalytical procedures applied before performing optical signal measurements, including cell lysis, antigen-antibody reaction, dye labelling or staining, can affect analysis and assay results.

4.2 Instruments and measurements

Photodetectors, including photomultipliers, photodiodes and image sensors, have specific spectral responsivities. Optical signals, including bioluminescence, chemiluminescence, fluorescence and absorption, can be measured accurately by using spectrally suited photodetectors and colour filters.

NOTE 1 Annex K gives examples for spectral responsivity data of photodetectors.

The optical signal measurement instruments are affected by environmental conditions, including laboratory temperature, and exposure to direct sunlight. Adjustment of the spatial resolution of an instrument can be required depending on the application.

Optical signals can be measured quantitatively when the signal intensity is within the dynamic range of the photodetector. Photodetectors have specific linear or nonlinear responsivities within this dynamic range, which can be determined with test measurements.

NOTE 2 The limits of linearity can be determined statistically.

Most instruments perform relative measurements of optical signals. The output values, therefore, depend on the instrument unless a reference material is available to establish a calibration curve. Only when the instruments are absolutely calibrated in radiometric values including the number of photons, can the measured optical signal values be expressed as absolute radiometric quantities.

Background signals can affect optical signal measurement results. Typical sources of background signals are electrical noises (e.g. dark count and read-out noise) and optical noises (e.g. stray light and external light).

Background signals can exist even in the absence of optical signals. Background signals are automatically or manually subtracted after optical signal measurement.

4.3 Optical references

Optical references can be used to confirm the performance of optical signal measurement instruments, including repeatability, intermediate precision, reproducibility, dynamic range and other related instrument performance.

Consistency of optical characteristics between the optical reference and the biological sample increases confidence in instrument performance and the analysis and assay results by measuring the optical signals of biological samples.

NOTE Examples for optical references are an LED-based reference light source (see Annex D for an exemplary luminometer qualification with LED), laser, slide of fluorescent glass, fluorescent dye in solution or other matrix (e.g. fluorescent bead), slide embedded fluorescent material, reference filter, reference cuvette, reference film, reference solution and power meter (see Annex B).

Optical references can be used for installation qualification, operational qualification and performance qualification. Optical references can also be used to compare photodetector responsivity between instruments.

Optical references can be used to calibrate optical signals to amounts or relative amounts, or potencies of target biological samples.

5 Minimum requirements to support optical signal measurement

5.1 Elements of photometric methods

Standardized approaches should be followed to provide accurate analysis and assay results by measuring the optical signal in photometric methods for analysis of biological samples.

Instruments, reagents, biological samples including cells, and other experimental materials used for optical signal measurement in the photometric methods shall be selected for the intended purpose and procedures. Reagents and biological samples shall be properly stored and maintained.

NOTE 1 Stability of reagents and biological samples can change during long-term storage and maintenance.

NOTE 2 In cells expressing reporter gene(s), including bioluminescence, chemiluminescence, fluorescence or colorimetric reporter gene, expression level of the reporter gene(s) can change during storage and subculturing. The copy number of the reporter gene(s) can also change during long-term subculturing.

The manufacturer's instructions should be followed for storage and use of reagents and biological samples.

Optical components of instruments (photodetectors, optics and light sources) used for optical signal measurements shall be selected in accordance with the optical characteristics, including spectral properties, of the photometric methods and biological sample.

Interference or enhancement of the optical signal by the apparatus, reagents, solvent and biological sample used should be taken into account.

NOTE 3 Some apparatus, reagents and solutions have inappropriate characteristics for optical signal measurements, including inhibition or enhancement of reactions creating interfering signals due to absorptive/fluorescent/quenching/phosphorescent/corrosive properties.

NOTE 4 Ancillary materials including phenol red (due to absorption) can alter optical signal measurement results.

NOTE 5 Adhesion to container walls can cause false concentration particularly for low concentration samples. Depending on instrument design, the concentration can also be too high due to carry over from a preceding measurement of high concentration samples.

5.2 Verification of optical signal measurement instruments

5.2.1 Optical references

Optical signal measurement instruments shall be verified using optical references.

NOTE 1 Examples of optical references and instruments are listed in Annex B.

Optical references can be used to verify repeatability, intermediate precision and reproducibility.

Optical signal measurement instruments that have been verified by the manufacturer should be maintained according to the manufacturer's instructions.

Reference light sources, including light emitting diode (LED) or laser, can be used to verify responsivity of luminometers, fluorescence plate readers, microarray readers and imaging analysers. Pulsed LEDs can be used as a reference light source for photodetectors in flow cytometers.

NOTE 2 Annex E gives information and an example for the application of reference light sources for comparison measurements of luminescent biological samples using luminometers.

Reference fluorescent materials, including fluorescent substance, solution or beads, can be used to verify responsivity of imaging analysers, flow cytometers, spectrofluorometers and fluorescence plate readers.

NOTE 3 Further guidance on characterization and assessment of suitable reference materials can be found in ISO Guide 35:2017.

A power meter (an optical power meter) can be used to verify excitation light power (W) of imaging analysers, flow cytometers, spectrofluorometers, microarray readers, fluorescence plate readers, spectrophotometers and plate readers.

A reference cuvette or reference material for absorbance measurement can be used to verify responsivity of plate readers, spectrophotometers and imaging analysers.

A reference light source whose optical signal value is calibrated absolutely against power (W) or number of photons can be applied to calibrate absolute sensitivity of the instruments.

NOTE 4 Annex J gives an example for the calibration of reference light sources and luminometers.

In-house standards (e.g. authentic materials) can be used as optical references when the reproducibility or the stability is confirmed.

5.2.2 Dynamic range

The dynamic range of the photodetector in an instrument to quantitatively measure optical signal(s) from biological sample(s) shall be determined.

NOTE 1 Evaluation of dynamic range is generally conducted as installation qualification and/or operational qualification of optical signal measurement instruments.

NOTE 2 The lower limit of dynamic range can be given by the limit of quantification, whereas the upper limit of dynamic range can be characterized by onset of unacceptable anomalies in sensitivity.

NOTE 3 Examples for the dynamic range determination of luminometers can be found in Annex G. Annex H gives an example for the construction of a calibration curve and the dynamic range determination of fluorescence plate readers. Annex I gives an example of the dynamic range determination for flow cytometers.

The dynamic range can be determined using an LED reference light source, serial dilution of reference material or reporter proteins, including bioluminescence, chemiluminescence, fluorescence and chromogenic proteins, chemiluminescent reagents, and reference cuvettes or reference materials for absorbance measurement.