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Nanotechnologies — Performance characteristics of nanosensors for chemical and biomolecule detection —

Part 1: Detection performance

Nanotechnologies — Caractéristiques de performance des nanocapteurs pour la détection de molécules chimiques et de biomolécules —

Partie 1: Performances de détection

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Foreword

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This document was prepared by Technical Committee ISO/TC 229 *Nanotechnologies*.

A list of all parts in the ISO 23367 series can be found on the ISO website.

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

Nanostructured materials possess fascinating properties such as high reactivity, excellent electrical conductivity, quantum-scaled confinement, great degree of biocompatibility, versatile optical transmission, outstanding electromagnetic properties and substantial surface area-to-volume ratio. Physical and chemical behaviour of nanomaterials are tunable by morphological variation as well as surface modification. It is these unique properties and functionalities that enable nanomaterials to be a novel solution for the development of advanced high-performance sensing devices.

Applications of advanced nanomaterials in the detection of chemicals or biomolecules have been widely studied to cater to variety of demands from innovative healthcare practice to industrial process improvement to potent environmental surveillance. By introducing nanotechnology in sample preparations, biomolecule reactions and chemical sensing procedures, the performance of traditional sensor technologies has been enhanced, brand-new nanosensors have been commercialized, and related markets have achieved remarkable growth.

There are several standards that specify the performance characteristics and the performance evaluation of a specific sensing or metrological device/equipment, especially in in vitro diagnosis, food safety management and environmental monitoring. Existing standards describe the performance of conventional sensors, however, there is not any standard document which generally addresses the enhanced sensing performance of a nanosensor. Moreover, the enhancement of sensing performance by nanotechnologies is not yet reflected in the existing relevant standards for conventional sensor technologies. As a result, there is a growing need for standardization to accurately describe the performance characteristics of nanosensors for chemical and biomolecule detection. This document standardizes performance characteristics of nanosensors which are utilized for performance evaluation in medical diagnosis, personal healthcare, environment monitoring, food quality and safety monitoring and biohazard defence.

Nanotechnologies — Performance characteristics of nanosensors for chemical and biomolecule detection —

Part 1: Detection performance

1 Scope

This document describes the performance characteristics necessary to evaluate the detection performance of nanosensors for chemical and biomolecule detection. This document does not cover the analytical performance characteristics or the performance evaluation procedure of a specific sensor.

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 99:2007, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*

ISO 16604:2004, *Clothing for protection against contact with blood and body fluids — Determination of resistance of protective clothing materials to penetration by blood-borne pathogens — Test method using Phi-X 174 bacteriophage*

ISO 17511:2020, *In vitro diagnostic medical devices — Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples*

ISO 18113-1:2022, *In vitro diagnostic medical devices — Information supplied by the manufacturer (labelling) — Part 1: Terms, definitions, and general requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/IEC Guide 99:2007, ISO 16604:2004, ISO 17511:2020, ISO 18113-1 and the following 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
analyte
component represented in the name of a measurable quantity

EXAMPLE In the type of quantity "mass of protein in 24-hour urine", "protein" is the analyte. In "amount of substance of glucose in plasma", "glucose" is the analyte. In both cases the long phrase represents the *measurand* (3.9).

[SOURCE: ISO 17511:2020, 3.1]

3.2
analytical performance
<nanosensor> ability of an assay using a nanosensor to measure or detect a particular analyte in a reference sample

3.3
assay
set of operations to determine the presence of or concentration of a particular component

[SOURCE: ISO 16604:2004, 3.2, modified — "analysis of a mixture" has been changed to "set of operations" and Note 1 to entry has been deleted.]

3.4
cut-off value
quantity value used as a limit to identify samples that indicate the presence or the absence of a specific disease, condition, or measurand

Note 1 to entry: The cut-off value defines which measurement results are reported as positive and which are reported as negative.

Note 2 to entry: Measurement results near the cut-off value can be inconclusive due to measurement uncertainty.

Note 3 to entry: The selection of the cut-off value determines the clinical specificity and clinical sensitivity of the examination.

[SOURCE: ISO 18113-1:2022, 3.2.15, modified — "decision limit" has been changed "limit" and Notes 1,2 and 3 to entry have been revised.]

3.5
detection performance
<nanosensor> ability of an assay using a nanosensor to determine the presence of a particular analyte above the cut-off value in a test sample

Note 1 to entry: A test sample is defined as a sample used to validate the performance of the nanosensor.

3.6
detection sensitivity
<nanosensor> ability of an assay using a nanosensor to recognize the presence of an analyte in a test sample

3.7
detection specificity
<nanosensor> ability of an assay using a nanosensor to recognize the absence of an analyte in a test sample

3.8
detection signal ratio
<nanosensor> ability of an assay using a nanosensor to clearly differentiate a positive response from a negative response

3.9
measurand
quantity intended to be measured

[SOURCE: ISO/IEC Guide 99:2007, 2.3, modified — Notes 1, 2, 3 and 4 to entry, and Examples 1 and 2 have been deleted.]

3.10
nanosensor
nano-enabled or nano-enhanced apparatus used to detect or identify events and changes in its environment, and transmit features of data to other electronics to convert them to a measurable output

3.11
performance characteristic
<nanosensor> parameter used to define the performance of a nanosensor

EXAMPLE Sensitivity, specificity and signal ratio.

Note 1 to entry: Information about more than one performance characteristic is usually required to evaluate the suitability of a nanosensor for its intended use.

3.12
performance evaluation
<nanosensor> investigation of a device or apparatus for the purpose of establishing or verifying its performance claims

4 Detection performance characteristics

4.1 General

Detection performance is the ability of an assay to discriminate between two subclasses of subjects, and detection performance evaluation of the assay using a nanosensor is intended mainly to detect the presence of a target chemical or biomolecule above a cut-off value. Examples of assays include the tests for infectious diseases in in vitro diagnosis,[1] food poisoning in food quality and safety monitoring,[2] defective product screening in quality control[3] and detection of biohazards in environmental monitoring.[4] In such cases, the assay is designed to distinguish between positive and negative responses, i.e. between results above or below a pre-determined cut-off value.

Typical detection performance characteristics for conventional sensors are detection sensitivity and detection specificity. However, there are limitations on existing detection performance characteristics to assess the enhanced performance of nanosensors as described in Annex A and Annex B. Therefore, detection performance characteristics for nanosensors need to be defined well enough to assess the enhancement of performance by nanotechnology as well as the reliability of the assay using nanosensors.

Clause 4 describes detection performance characteristics of nanosensor for chemical and biomolecule detection that shall be essentially evaluated using test samples.

4.2 Detection sensitivity

Detection sensitivity is the probability of the assay scoring positive in test samples coming from subjects known to have the analyte. Detection sensitivity is expressed as the ratio of true positive samples over the total number of samples which should give positive results, i.e. true positive samples plus false negative samples. This performance characteristic may be expressed as percentage, after multiplication by 100.

$$D_{\text{sens}} = \frac{N_{\text{TP}}}{N_{\text{TP}} + N_{\text{FN}}}$$

where

D_{sens} is the detection sensitivity;

N_{TP} is the number of true positives;

N_{FN} is the number of false negatives.

4.3 Detection specificity

Detection specificity is the probability of the assay scoring negative in test samples coming from subjects known to be free of the analyte. Detection specificity is expressed as the ratio of the true negative samples over the total number of samples which should give negative results, i.e. true negative plus false positive samples. This performance characteristic may be expressed as percentage, after multiplication by 100.

$$D_{\text{spec}} = \frac{N_{\text{TN}}}{N_{\text{TN}} + N_{\text{FP}}}$$

where

D_{spec} is the detection sensitivity;

N_{TN} is the number of true negatives;

N_{FP} is the number of false positives.

4.4 Detection signal ratio

Detection signal ratio is the ability of an assay to clearly differentiate a positive response from a negative response in test samples. Detection signal ratio is expressed as the ratio of the averaged signal intensity of true positive samples over the averaged signal intensity of the true negative samples.

$$R_{D\text{signal}} = \frac{I_{\text{averaged,TP}}}{I_{\text{averaged,TN}}}$$

where

$R_{D\text{signal}}$ is the detection signal ratio;

$I_{\text{average,TP}}$ is the averaged signal intensity of true positive samples;

$I_{\text{average,TN}}$ is the averaged signal intensity of the true negative samples.

Annex A
(informative)

Performance evaluation of a sensor

A.1 General

Sensors are used to determine the presence of chemical or biological substances and to express them quantitatively. In particular, while the purpose of a measuring instrument or an analyser is limited to a quantitative expression of a physicochemical quantity, the sensor serves to provide data for judgment related to the existence of a substance according to the user's intention. As a representative example, a sensor developed for medical purposes is used as a diagnostic device, and is manufactured to confirm the presence of a pathogen or biomarker, and to indicate the presence or absence of a disease according to the result. Therefore, the sensor as an in vitro diagnostic device should be evaluated whether it has performance characteristics such as sensitivity and specificity that meet the requirements for diagnosing diseases. In applications such as water/air purification systems or environmental pollution monitoring, the performance of a sensor is evaluated according to the purpose of determining the presence or absence of residual pollutants, and to the ability to quantify the amount of residual pollutants.

The performance evaluation of a sensor proceeds as a verification procedure at the development stage or a validation procedure at the post-development stage. While often used intermixed, verification and validation are quite different procedures with different goals and different means to achieve those goals. Verification is defined as confirmation, through the provision of objective evidence, that specified requirements have been fulfilled, and validation is defined as confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. [5] Here, the requirement for a specific intended use or application refers to the correct determination on the presence or absence of a disease in the field of in vitro diagnostics, residual pollutants in the water/air purification system, and environmental hazards in the environmental pollution monitoring.

A.2 Analytical versus detection performance evaluation

In the verification procedure, performance evaluation must confirm whether the sensor is manufactured in accordance with the designed requirements, and an analytical performance evaluation method is used. The analytical performance evaluation refers to how well a sensor can measure the analyte of interest – in other words, accurate and reproducible. In the analytical performance evaluation, performance characteristics, such as analytical sensitivity, analytical specificity, accuracy, precision, repeatability, reproducibility, limit of detection, limit of quatification, etc., are evaluated using a reference sample having a measured value with a uncertainty.

During the validation procedure, in contrast, performance evaluation must confirm whether the sensor operates according to its intended use, and a detection performance evaluation method is used. The detection performance evaluation refers to how well a given test can discriminate the presence or absence of the analyte. In the detection performance evaluation, the test sample is not a reference sample or any artificial buffered sample, but a natural unbuffered sample in which unexpected impurities may co-exist causing false response. Therefore, the test results obtained must be compared with the results by a “gold standard“ method to evaluate the detection performance characteristics, such as detection sensitivity, detection specificity and detection signal ratio. A comparison of the two performance evaluation methods is summarized in Table A.1.

Table A.1 — Analytical versus detection performance evaluation

Evaluation	Analytical performance	Detection peformance
purpose	verification procedure	validation procedure
test sample	artificial, buffered	natural, unbuffered
reference criteria	certified value of reference sample	test result by gold standard
characteristics	analytical sensitivity, analytical specificity, accuracy, precision, repeatability, reproducibility, limit of detection, limit of quantification, etc.	detection sensitivity, detection specificity and detection signal ratio

Annex B
(informative)

Limitations of existing detection performance characteristics on nanosensor

B.1 General

The performance of nanosensors is divided into analytical performance and detection performance. The analytical performances are evaluated during the verification test of nanosensors that measure or detect analyte in reference samples, and the detection performance is evaluated during validation test using test samples for decision-making purposes such as medical diagnosis, quality control and monitoring system.

During analytical performance evaluation, the analytical performance characteristics such as sensitivity, selectivity, limit of detection, limit of determination, limit of qantification, repeatability, reproducibility, stability, signal-to-noise, etc., as defined in the literatures are evaluated.[6]-[8] In the analytical performance characteristics measured with continuous values, the enhanced performance by nanotechnology can be well evaluated. However, it is difficult to clearly evaluate the enhanced performance of the nanosensors by using the detection performacne characteristics based on binary classification. For a clearer understanding, Clause B.2 describes binary classification. The difficulty of evaluating the detection performance of nanosensors is described in Clause B.3.

B.2 Binary classification

Binary classification is the task of classifying elements of a given set into two groups according to classification rules (determining which group each group belongs to). The presence of disease in medical diagnostics, determination of pass/fail in quality control, and safe/alert announcements in surveillance systems are examples of typical binary classifications.

As with most measurements, tests with continuous values can be artificially made binary by defining a cut-off value, and the test result is positive/pass/safe or negative/fail/alert, depending on whether the result is higher or lower than the cut-off.

Given a classification of a specific data set, there are four basic combinations of actual sample category and test outcome category, shown in Table B.1: true positives (correct positive assignments), true negatives (correct negative assignments), false positives (incorrect positive assignments) and false negatives (incorrect negative assignments).

Table B.1 — Four basic combinations of binary classification

Test outcome	Positive sample	Negative sample
Positive	True positive	False positive
Negative	False negative	True negative

False positives and false negatives are caused by the presence of other materials in the sample such as matrices, impurities, interfering substances or inhibitors which may affect the sensing performance of the nanosensors.

In vitro diagnosis to check the presence of disease, food quality and safety monitoring in food manufacturing to determine the occurrence of pathogen and environmental surveillance systems to alert the level of biohazard such as fine dust in air or toxins in water are good examples to adopt binary

classification in industries. In addition, these applications are also actively utilizing nano-sensors with improved performance.

As an example of binary classification, there is Pass/Fail classification in food quality and safety monitoring. Food poisoning bacteria detected during food manufacturing process can be tolerated within the usual range (Pass), but if food poisoning bacteria are detected exceeding the cut-off value (Fail), the food manufacturing process should be stopped and the produced food must be disposed for safety. The improved performance of nanosensors can enhance reliability in stakeholders‘ risk management causing disruption of manufacturing processes and disposal of produced food. Also, the safe/warning/alarm classifications of fine-dust or toxin in environment monitoring are an advanced example of binary classifications. The government's preparedness strategy to ensure public safety must be announced, depending on the detected classification of fine-dust or toxin, and the improved performance of nanosensors can impart strong credibility to the government's crisis management capabilities.

B.3 Example of detection performance evaluation

The performance of a conventional sensor (Sensor A) and a nanosensor (Sensor B) is first compared using existing performance characteristics. After the measurements of 14 samples, respectively, the results in Figure B.1 show that Sensor A and Sensor B, all have six true positives and one false negative for seven positive samples, and seven true negatives for seven negative samples. Both sensors perform with 85,7 % sensitivity and 100 % specificity. It may therefore conclude that the performance of these two very different sensors are the same as each other.

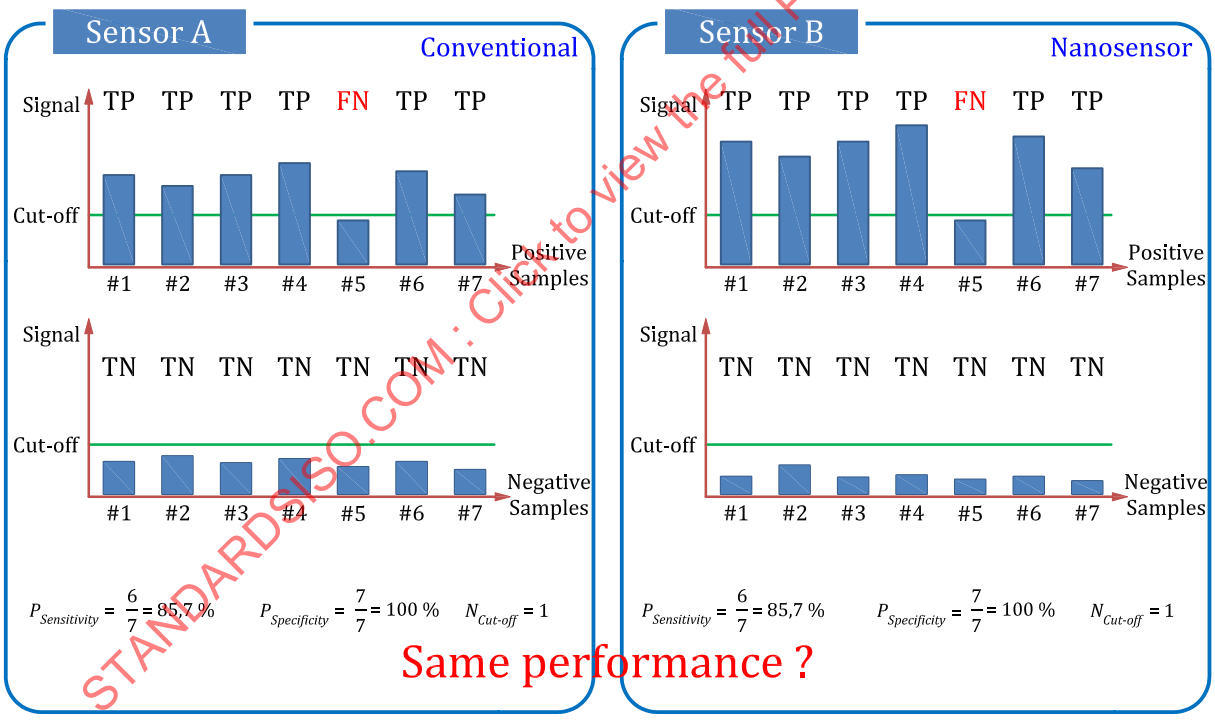

Figure B.1 — Detection performance evaluation using sensitivity and specificity

If the signal ratio performance characteristics are compared in addition to the existing sensitivity and specificity, the detection signal ratio of the conventional sensor and the nanosensor are calculated to be 2,768 and 6,569, respectively, and the stakeholders will determine that the nanosensor performance is better and there is the performance enhancement by nanotechnology shown in Figure B.2.