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Introduction

This draft standard is based on national and international discussions. Your comments on this draft are invited and will assist in the preparation of the consequent standard.

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UK Vote

Please indicate whether you consider the UK should submit a negative (with supporting technical reasons) or positive vote on this draft. Please indicate if you are aware of any reason why this draft standard should not be published as a British Standard.

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Template for comments and secretariat observations

Date: xx/xx/20xx	Document: ISO/DIS xxxx
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1	2	(3)	4	5	(6)	7
MB	Clause No./ Subclause No./Annex (e.g. 3.1)	Paragraph/ Figure/Table/Note	Type of comment	Comment (justification for change) by the MB	Proposed change by the MB	Secretariat observations on each comment submitted
	3.1	Definition 1	ed	Definition is ambiguous and needs clarifying.	Amend to read '...so that the mains connector to which no connection...'	
	6.4	Paragraph 2	te	The use of the UV photometer as an alternative cannot be supported as serious problems have been encountered in its use in the UK.	Delete reference to UV photometer.	



DRAFT International Standard

ISO/DIS 16921-2

Biotechnology — Gene delivery systems —

Part 2: Guide for quantification methods for viral vectors

ICS: 07.080

ISO/TC 276/SC 1

Secretariat: **ANSI**

Voting begins on:
2025-01-14

Voting terminates on:
2025-04-08

This document is circulated as received from the committee secretariat.

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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).

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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*, Subcommittee SC 1, *Analytical methods*.

A list of all parts in the ISO 16921 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

Modern and emerging biotechnology is underpinned by the ability to manipulate the genes and genomes of living systems. Gene delivery systems are foundational to genome engineering. Gene delivery technology is evolving rapidly with numerous types of gene delivery systems providing a comprehensive set of tools and capabilities for *in vitro* or *in vivo* targeted delivery.

The ISO 16921 series consists of multiple parts to provide common understanding, guides, analytical methods and, data reporting for characterizing these emerging biotechnology tools. Part 1 of this series focuses on Vocabulary related to gene delivery systems. Part 2 focuses on quantification of one type of gene delivery system, viral vectors. Additional parts will be developed to focus on measurements for other gene delivery systems.

Viral vectors are engineered viruses for delivering the desired payload into target cells. Viral vectors are powerful molecular biology tools and have been increasingly used in broad biotechnology applications and products. Various types of viral vectors are used as advanced gene therapies, as vaccines, and as critical reagents for cellular therapies. They have also been increasingly used in genome editing applications. Viral vector titer (titre) is central to all applications. Robust measurements for the quantification and reporting of viral vector titer are important for the industry. This document provides general guidance for viral vector titer as well as aspects of functional analysis including method selection, sample preparation, measurement, qualification and validation, data analysis and reporting.

Biotechnology — Gene delivery systems —

Part 2: Guide for quantification methods for viral vectors

1 Scope

This document provides minimum requirements for quantifying viral vectors in terms of physical titer and their associated activity. It provides key considerations for quantification methods for viral vector titer as well as activity, including method selection, measurement process, data analysis, and reporting.

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/IS 16921-1, *Biotechnology — Gene Delivery Systems — Part 1: Vocabulary*

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 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

direct particle counting

counting method in which one signal is (or several signals are) detected for each single event

Note 1 to entry: Each single event should represent a single viral particle in an idealized measurement

[SOURCE: ISO 20391-1:2018(en), 3.12]

3.2

indirect particle counting

counting method during which a signal (or a set of signals) is measured from a population of viral particles and that signal is then related to viral titer based on a measurement-specific mathematical model (e.g. calibration curve)

[SOURCE: ISO 20391-1:2018(en), 3.13]

4 General concepts for viral vector titer

Viral vectors are laboratory modified viruses designed to deliver nucleic acid into a cell. As such, viral vectors are almost always replication incompetent, so that no new viral vectors can be produced within the target cell.

NOTE 1 Viral vectors can be made to be replication competent in the presence of a helper virus or complementary cell.

NOTE 2 Replication competent viral vectors are generally not used in therapeutical applications due to safety concerns.

A functional viral vector generally consists of three components:

- the protein capsid with or without the envelope that encapsulates the payload, and defines the vector's tissue or cell tropism and antigen recognition;
- the gene of interest, which when expressed in cells, serves to confer a desired functional outcome; and
- the regulatory components including the combined enhancer, promoter, and auxiliary elements that control stable or transient somatic expression of the transgene as an episome or as a chromosomal integrant.

NOTE 3 Some viral vectors designs do not precisely follow the transgene and regulatory cassette descriptions stated above. Examples include those utilizing micro RNAs, guide RNAs, multiple transgenes/promoters, and dual vectors (which utilize *in vivo* splicing).

The process to engineer and manufacture viral vectors is complex and evolving. Current technologies generally produce a mixture of functional viral vectors that can deliver the appropriate payload into the target cells as well as non-functional viral vectors and free capsid proteins ([Figure 1](#)).

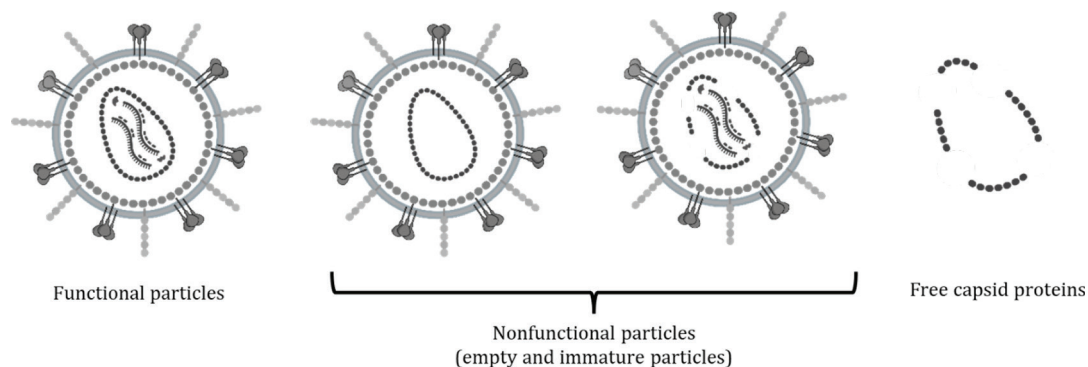


Figure 1 — Products from viral vector production that include functional particles, nonfunctional particles, and free capsid protein. Adapted from “Understanding viral titration- behind the science”^[1].

Non-functional viral vector particles do not have all the necessary components to complete the transduction process, express the intended protein, and result in the functional outcome. Non-functional viral vector particles include various types of defects, including for example:

- vectors with defective capsids that may affect its tissue or cell tropism and antigen recognition,
- vectors that contain missing or defective payloads,
- vectors that contain missing or defective regulatory components.

General methods for 1) quantification of physical titer of viral vectors and 2) functional characterization of viral vectors using cell-based measurements are shown in [figure 2](#).

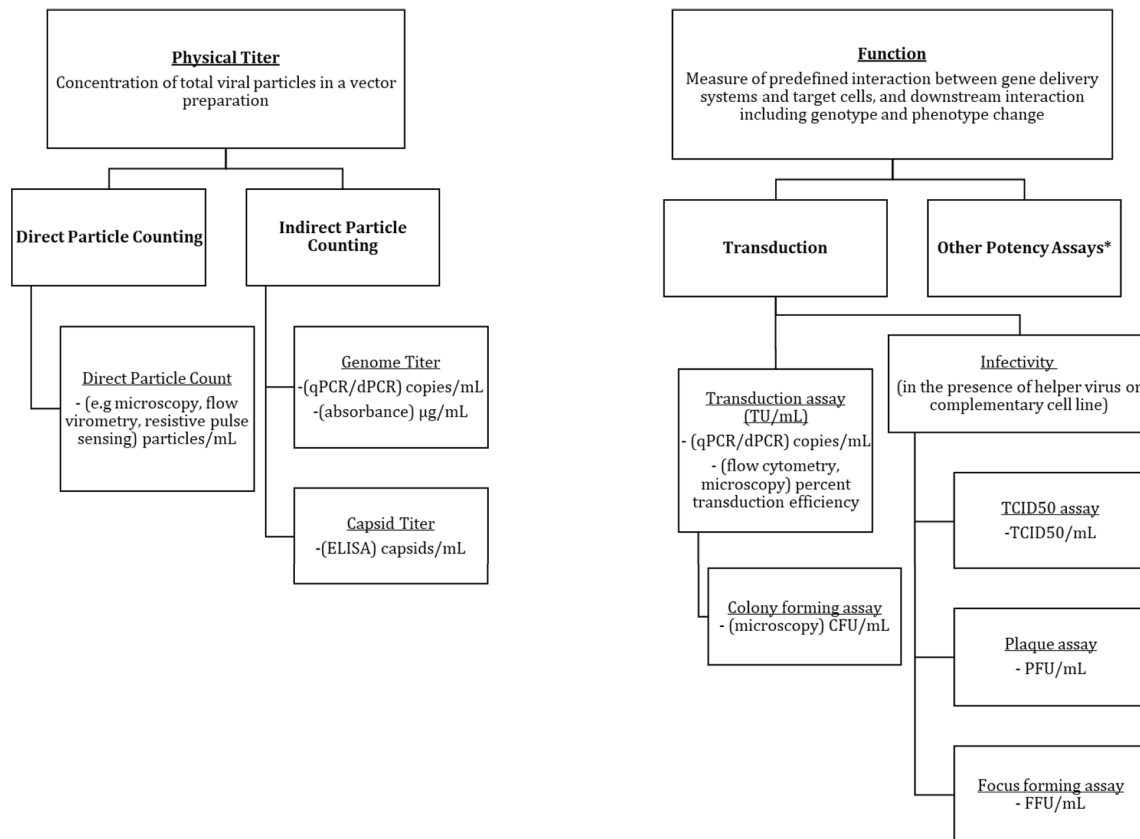


Figure 2 — Analytical methods for general characterization of viral vectors and their interactions with cells. *Additional potency assays for titer quantification are often specific to the product and mechanism of action and is beyond the scope of this document

Physical titer of viral vectors refers to the particle count, which can be determined by direct particle counting or indirect particle counting methods.

Direct particle counting involves the recording of a signal or a set of signals from individual viral particles (3.1). Direct particle counting requires well-dispersed particles for optimal performance. The presence of debris and aggregated or agglomerated particle may lead to over- or underestimated virus titer. Whenever possible, a process should be established to prepare well- dispersed samples with minimized debris, aggregate, and agglomerate content.

Indirect particle counting methods use a surrogate measure to evaluate the count. The accuracy of these methods depends on the accuracy of the measurement and the accuracy of a calibration curve, if a calibration curve is needed. Indirect particle counting methods include:

- the use of a calibration to estimate a count (e.g. fluorescence, qPCR, ELISA, or another method to back calculate count using a calibration curve),
- the measuring a component of the particle to infer the count (e.g. quantifying the total capsid protein concentration to estimate particle count).

Activity methods are used to measure the interaction between viral vector particles and target cells. Activity measures determine the concentration of functional particles in a viral vector preparation. Activity measurements can be further divided into transduction and other potency measures to gain insight into general transduction activity and product specific potency.

Transduction assays measure viral vector transgene entry into target cells for non-replication competent viral systems. Vector copy number per cell or transduction efficiency can be measured in the target cells. A dilution series of virus to cells is generally used to specify at which dilutions the multiplicity of infection to

transduction efficiency is linear. The transduction units in the original virus preparation can be determined from the specified range of the dilution series.

Infectivity assays may include a viral vector, a helper virus, and a complementary cell line that enables the viral vector to replicate. Viral replication can result in target cells undergoing cytopathic effect. Replication and cytopathic effect can be visualized and quantified as plaques or foci in the cells. Viral vector replication in target cells enables quantification of virus activity and infectivity assay titer.

Potency assays measure transgene expression and transgene activity. Transduction assays can be considered one part of potency measures which can measure transgene insertion, integration, and expression. Additional potency measures beyond transduction and transgene expression are often production specific measures of transgene mechanism of action. Due to the product specific nature of such assays, use of potency assays for viral vector quantification are beyond the scope of this document.

See [Annex A](#) for table of attributes, biological properties, analytical methods, measurement principles, and units for the quantification of viral vector titer.

4.1 Physical titer measurements

Physical titer refers to the total concentration of viral vectors or the concentration of a predefined, subset of viral vectors.

Physical titer of viral vectors shall be expressed as concentration per volume (1/mL).

Physical titer can be determined via several analytical methods, including direct particle counting methods and indirect particle counting methods.

An appropriate measurement shall be selected to determine the total quantity or the quantity of a predefined, subset of viral vectors.

NOTE Assessment of the quality of the viral vectors may involve the use of two or more titers, such as filled to empty ratio.

See [Annex B](#) for additional physical titer methods.

4.1.1 Direct viral vector titer measurement

Direct viral vector titer methods identify and enumerate single particles. Direct viral vector titer can be used for total viral vector enumeration or enumeration of particles of a specific predefined attribute.

NOTE Direct viral vector titer measurements can include electron microscopy-based counting, flow virometry, resistive pulse sensing, nanoparticle tracking analysis, and other methods capable of capturing single entities (i.e., viral vector particles).

Results of direct particle counting methods shall be reported in terms of particles/mL or specified units of volume.

Due to sample heterogeneity, direct viral vector counting methods may inadvertently count impurities, such as extracellular vesicles, as viral vectors. Counting methods targeting only the viral vectors within a sample may therefore be more appropriate. Combining methods specific for impurities, such as flow cytometry that targets extracellular vesicles, can also be used to correct direct particle count measurements to be more accurate for viral particles.

4.1.2 Indirect viral vector titer measurement

Indirect viral vector titer measurements include all methods to extrapolate the viral vector quantity not associated with enumeration.

Indirect methods generally involve the use of a calibration curve to determine the quantity. These include methods that quantifies a defined attribute of the viral vector, such as capsid protein quantification and genome quantification methods.