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Biotechnology — Cell counting —

Part 2: Experimental design and statistical analysis to quantify counting method performance

Biotechnologie — Dénombrement des cellules —

Partie 2: Conception expérimentale et analyse statistique pour quantifier les performances de la méthode de dénombrement

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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).

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.*

A list of all parts in the ISO 20391 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

Cell counting impacts many aspects of biotechnology, from biomanufacturing to medical diagnosis and advanced therapy. The cell count can serve as an in-process quality control or be used in decision-making. Cell count is also an important parameter in many cell-based assays, including activity and potency, which are often normalized to the cell count to allow data comparison.

Cell count is generally expressed as a concentration and can reflect the total cell count of a cell population (total cell count) or subpopulation (differential cell count). Advances in instrumentation have resulted in a wide range of cell counting techniques/instruments for total and/or differential cell counts. In the absence of a readily available reference material or ground truth, the accuracy of a measurement method has been difficult to ascertain. This has been confounded by the complexity of the biological preparation (e.g. cell type, sources, preparation, etc.). Several standards that address sector/application-specific cell counting or the use of a specific measurement system exist (See ISO 20391-1 and Reference [16] for further information). Some of these methods use a comparability approach whereby the result from a newer cell counting test method is traced to the results obtained from a more established cell counting method. While the comparability approach allows the data from the second instrument to be benchmarked against those obtained from a primary (more established) instrument, it does not address the quality of either measurement process[17]. There remains a need to develop strategies to provide assurance for the quality of a cell counting measurement process in the absence of a reference material or reference method[17].

This document provides a method for evaluating aspects of the quality of a cell counting measurement process through the use of a dilution series experimental design. From this experimental design, a set of quality indicators are derived to assess the performance of a cell counting measurement process. Specifically, the quality indicators assess precision and proportionality of cell counting measurement processes. This approach is particularly useful when accuracy cannot be determined (i.e. in the absence of a traceable reference method or traceable reference material) and is also relevant in aspects of validating and monitoring the quality of cell counting measurement processes in general[17].

Information in this document is intended to provide confidence in the data produced by a chosen cell counting measurement process. This approach can be useful for selecting or optimizing a measurement process for a given cell preparation. This approach can also provide supporting performance parameters that can be utilized during performance qualification of a particular cell counting measurement process.

Biotechnology — Cell counting —

Part 2: Experimental design and statistical analysis to quantify counting method performance

1 Scope

This document provides a method for evaluating aspects of the quality of a cell counting measurement process for a specific cell preparation through a set of quality indicators derived from a dilution series experimental design and statistical analysis. The quality indicators are based on repeatability of the measurement and the degree to which the results conform to an ideal proportional response to dilution. This method is applicable to total, differential, direct and indirect cell counting measurement processes, provided that the measurement process meets the criteria of the experimental design (e.g. cells are suspended in a solution).

This method is most suitable during cell counting method development, optimization, validation, evaluation and/or verification of cell counting measurement processes.

This method is especially applicable in cases where an appropriate reference material to assess accuracy is not readily available. This method does not directly provide the accuracy of the cell count.

This method is primarily applicable to eukaryotic cells.

NOTE Several sector/application specific international and national standards for cell counting exist. Where applicable, consulting existing standards when operating within their scope can be helpful.

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 20391-1, *Biotechnology — Cell counting — Part 1: General guidance on cell counting methods*

3 Terms, definitions, symbols and abbreviated terms

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 http://www.electropedia.org/

3.1 Terms and definitions

3.1.1
accuracy
<measurement> closeness of agreement between a measured quantity value and a true quantity value of a measurand

[SOURCE: ISO/IEC Guide 99:2007, 2.13, modified — Notes deleted]