First edition
2019-08

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

Reference number
ISO 20391-2:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword ... **v**

Introduction ... **vi**

1 Scope ... **1**

2 Normative references ... **1**

3 Terms, definitions, symbols and abbreviated terms ... **1**
3.1 Terms and definitions ... 1
3.2 List of abbreviated terms and symbols ... 7

4 Principle ... **8**
4.1 General ... 8
4.2 Proportionality ... 9
4.3 Deviation from proportionality ... 9

5 Experimental design ... **10**
5.1 General ... 10
5.2 Considerations for the cell counting measurement process ... 10
5.3 Preparation of samples for the experimental design ... 11
5.3.1 General ... 11
5.3.2 Stock cell solution ... 11
5.3.3 Dilution fraction experimental design ... 12
5.3.4 Considerations for generating dilution fractions ... 13
5.4 Test sample labelling ... 14
5.5 Measurement of the test sample ... 14

6 Statistical methods ... **15**
6.1 General ... 15
6.2 Mean cell count ... 16
6.3 Measurement precision ... 16
6.4 Proportional model fit ... 16
6.5 Coefficient of determination ... 17
6.6 Proportionality index (*PI*) ... 17
6.6.1 General ... 17
6.6.2 Calculation of the smoothed residual (e_{smoothed}) ... 18
6.6.3 Calculation of proportionality index (*PI*) ... 18
6.7 Additional statistical analysis and quality metrics ... 19
6.8 Data interpretation ... 19
6.8.1 General ... 19
6.8.2 Interpretation of %*CV* ... 19
6.8.3 Interpretation of R^2 ... 19
6.8.4 Interpretation of *PI* values ... 20
6.8.5 Comparison of *PI* values ... 20

7 Reporting ... **20**
7.1 Reporting of quality indicators ... 20
7.2 Documentation of experimental design parameters and statistical analysis method ... 21
7.3 Additional reporting elements on the cell counting measurement process ... 22

Annex A (informative) **Method to assess pipetting error contributions to dilution integrity** ... **23**

Annex B (normative) **Method to calculate smoothed residual** (e_{smoothed}) **when a set of measured dilution fractions** (DF_{measured}) **is obtained** ... **27**

Annex C (informative) **Example formulae for calculating** *PI* ... **29**

Annex D (informative) **Use case 1 — Evaluating the quality of a single cell counting measurement process** ... **31**

Annex E (informative) **Use case 2 — Comparing the quality of several cell counting measurement processes** **38**

Bibliography **52**

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.