

ISO 20391-2:2019 (E)

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

Contents

	Foreword
	Introduction
1	Scope
2	Normative references
3	Terms, definitions, symbols and abbreviated terms
3.1	Terms and definitions
3.2	List of abbreviated terms and symbols
4	Principle
4.1	General
4.2	Proportionality
4.3	Deviation from proportionality
5	Experimental design
5.1	General
5.2	Considerations for the cell counting measurement process
5.3	Preparation of samples for the experimental design
5.3.1	General
5.3.2	Stock cell solution
5.3.3	Dilution fraction experimental design
5.3.4	Considerations for generating dilution fractions
5.4	Test sample labelling
5.5	Measurement of the test sample
6	Statistical methods
6.1	General
6.2	Mean cell count
6.3	Measurement precision
6.4	Proportional model fit
6.5	Coefficient of determination
6.6	Proportionality index (PI)
6.6.1	General
6.6.2	Calculation of the smoothed residual (esmoothed)
6.6.3	Calculation of proportionality index (PI)
6.7	Additional statistical analysis and quality metrics
6.8	Data interpretation
6.8.1	General
6.8.2	Interpretation of %CV
6.8.3	Interpretation of R2
6.8.4	Interpretation of PI values
6.8.5	Comparison of PI values
7	Reporting
7.1	Reporting of quality indicators
7.2	Documentation of experimental design parameters and statistical analysis method
7.3	Additional reporting elements on the cell counting measurement process
Annex A	(informative) Method to assess pipetting error contributions to dilution integrity
A.1	General

- A.2 Example procedure for pre-evaluating pipetting error contributions to dilution integrity using a calibrated scale to obtain accurate volume estimates upon pipetting
 - A.3 Example procedure for obtaining a measured DF during the evaluation of PI using a calibrated scale to obtain accurate volume estimates
- Annex B (normative) Method to calculate smoothed residual (esmoothed) when a set of measured dilution fractions (DF_{measured}) is obtained**
- Annex C (informative) Example formulae for calculating PI**
- C.1 Detailed example of the calculation of PI based on sum of the absolute value of scaled smoothed residuals
 - C.2 Detailed example of the calculation of PI based on R² of smoothed residuals
 - C.3 Additional examples of the calculation of PI when measured dilution fraction is utilized
- Annex D (informative) Use case 1 — Evaluating the quality of a single cell counting measurement process**
- D.1 General
 - D.2 Description of experimental design and statistical analysis
 - D.3 Raw data and data analysis for use case 1
 - D.4 Example report for use case 1
 - D.5 Interpretation
- Annex E (informative) Use case 2 — Comparing the quality of several cell counting measurement processes**
- E.1 General
 - E.2 Description of experimental design and statistical analysis
 - E.3 Raw data and data analysis for use case 2
 - E.4 Example report for use case 2
 - E.5 Comparison of cell counting methods evaluated in use case 2
 - E.5.1 Comparing quality indicators between cell counting methods
 - E.6 Interpretation
 - E.7 Consistency of results with underlying simulated model

Page count: 53