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Evaluating the quality of a cell counting measurement process via a dilution series experimental design

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Abstract

Background aims. Cell counting measurements are critical in the research, development and manufacturing of cell-based products, yet determining cell quantity with accuracy and precision remains a challenge. Validating and evaluating a cell counting measurement process can be difficult because of the lack of appropriate reference material. Here we describe an experimental design and statistical analysis approach to evaluate the quality of a cell counting measurement process in the absence of appropriate reference materials or reference methods. *Methods.* The experimental design is based on a dilution series study with replicate samples and observations as well as measurement process controls. The statistical analysis evaluates the precision and proportionality of the cell counting measurement process and can be used to compare the quality of two or more counting methods. As an illustration of this approach, cell counting measurement processes (automated and manual methods) were compared for a human mesenchymal stromal cell (hMSC) preparation. *Results.* For the hMSC preparation investigated, results indicated that the automated method performed better than the manual counting methods in terms of precision and proportionality. *Discussion.* By conducting well controlled dilution series experimental designs coupled with appropriate statistical analysis, quantitative indicators of repeatability and proportionality can be calculated to provide an assessment of cell counting measurement quality. This approach does not rely on the use of a reference material or comparison to "gold standard" methods known to have limited assurance of accuracy and precision. The approach presented here may help the selection, optimization, and/or validation of a cell counting measurement process.

Key Words: *cell counting, experimental design, measurement assurance, statistical analysis*

Introduction

Cell counting is a fundamental measurement in biotechnology and is used routinely in research and development, cell-based manufacturing and the release of cell-based therapeutics. The cell quantity (generally expressed as concentration for a cell suspension) can serve as an in-process quality control or be used in release assays [1,2]. Cell quantity is also an important parameter in many cell-based activity and potency assays, which are often normalized to the cell quantity to enable data comparison [3,4]. In these and other cases, an error in cell counting will propagate through subsequent measurements to affect the overall quality of bioassay results [5]. Despite its common use, counting of cells with high measurement confidence (accuracy and precision) remains a challenge [6].

Many cell counting methods exist that detect and enumerate cells for a given volume (or area), either manually or via an automated process [7–11]. Difficulties in cell counting arise from the complex biological and dynamic properties of cells. For example, a given cell preparation may contain aggregated cells, cells of varying shapes and sizes, mixed cell populations, cells in different stages of growth or death and/or debris from matrix components or cell fragments. The cell counting measurement process may include several preparation steps that can introduce variability [12]. For example, a dilution or sampling step to obtain appropriate volumes and concentrations of cells for a particular measurement may introduce unintended error. As another example, procedures to reduce the heterogeneity in a cell preparation (e.g., mixing, disaggregation via lysis buffers) and to help identify specific cell sub-populations (e.g., cell staining) may alter the cell preparation in systematic or random ways, reducing its representativeness of the broader cell population and leading to inaccurate interpretations of measurement results.

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An ideal cell counting method would be accurate (i.e., close to the true cell concentration) as well as precise (i.e., repeatable with low variability). The precision of a cell counting measurement may be evaluated by replicate observations of the same cell sample. However, high precision does not ensure high accuracy due to potential presence of bias. Assessing the accuracy for cell counting has been challenging. Accuracy is ideally evaluated through the use of a reference material with a known "true" value or via a reference method [13]. It has been difficult to develop cell-based reference materials that are stable, homogenous and fit-for-purpose [14]. Reference methods have been developed for the enumeration of blood cells through well calibrated flow cytometry measurements [15]; however, these methods are not yet applicable to a wide range cell counting measurements. In the absence of an appropriate reference material or reference method, some have used a benchmark method to enable comparability [16]. In many cases, the manual counting chamber (i.e., hemocytometer) is used as the "gold standard." It is broadly recognized, however, that manual counting methods are generally less precise than automated counting methods, and the accuracy of the manual counting method itself cannot be established [7,8,17,18].

Here, we describe an alternative method to assess the quality of a cell counting measurement in the absence of an appropriate reference material, via a dilution experiment. The method is based on adherence to the fundamental principle of proportionality: that the concentration computed from a true count would scale proportionally with dilution fraction. Proportionality is achieved when the ratio of measured cell quantity to true cell quantity is constant across a given range of cell concentration. Because true cell quantity is unknown, dilution fraction is used as a surrogate to monitor proportionality. Proportionality is observed when a change in dilution fraction is accompanied by a proportional change (i.e., related by a constant multiplier) in the measured cell quantity (Figure 1). Proportionality must hold true for an accurate cell counting measurement process and deviations from proportionality indicate the presence of an error (systematic or random). In this regard, well-controlled dilution fractions serve as an internal control to evaluate accuracy relative to the ideal proportional response between cell counts across the dilution fractions (Supplement 1). Quality of a cell counting measurement is then related to this inherent principle to which all cell counting methods should adhere regardless of the measurement platform or cell type. The experimental design examines multiple well-controlled dilution fractions of a cell suspension with replicate samples and replicate observations within the intended concentration range of use, to enable assessments of both precision and proportionality. This experimental design to characterize the quality of a cell counting measurement process enables decision-making through fit-for-purpose statistical analysis. We illustrate the utility of this approach in a case study evaluating total cell concentration obtained via manual counting and automated counting for human mesenchymal stromal cell (hMSC) preparations.

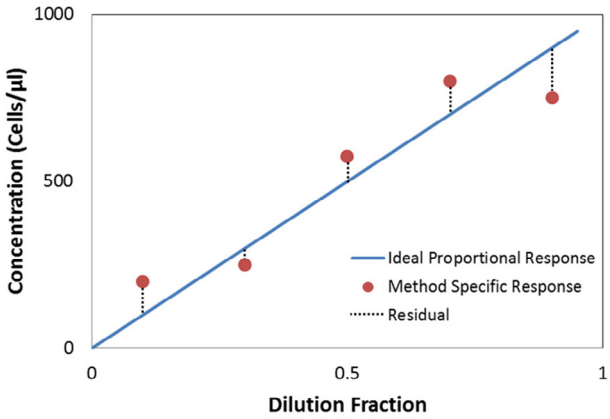

Figure 1. Schematic of a hypothetical cell counting dilution series experiment. Red dots represent collected data, and the blue line represents the proportional model fit to the data. Dotted lines represent the residuals that can be calculated between the data and the model fit.

Methods[1]

Evaluating quality of a cell counting measurement process

Precision and proportionality to dilution are identified as fundamental properties of a cell counting method that should hold true for a method to be accurate and useful in subsequent decision-making. A dilution series experimental design was developed to evaluate the precision and proportionality of cell counting methods. In the absence of a reference material, these metrics may serve as an assessment of cell counting method quality. This quality assessment evaluates the full cell counting measurement process, including aspects of sampling and sample preparation, which may introduce variability into a cell counting result.

Principle of proportionality

In an ideal cell counting method, cell concentration divided by dilution fraction is constant across samples

[1]Certain commercial equipment, instruments or materials are identified in this paper to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.