



## Strategies for Achieving Measurement Assurance for Cell Therapy Products

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**Key Words.** Biological assay • Cell- and tissue-based therapy • Quality control • Reference standards

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Received September 28, 2015; accepted for publication January 18, 2016; published Online First on April 21, 2016.

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1066-5099/2016/\$20.00/0

[http://dx.doi.org/  
10.5966/sctm.2015-0269](http://dx.doi.org/10.5966/sctm.2015-0269)

### ABSTRACT

The cell therapy industry has identified the inability to reliably characterize cells as possibly its greatest challenge and has called for standards and reference materials to provide assurance for measurements of cell properties. The challenges in characterization of cell therapy products can be largely addressed with systematic approaches for assessing sources of uncertainty and improving confidence in key measurements. This article presents the many strategies that can be used to ensure measurement confidence and discusses them in terms of how they can be applied to characterization of cell therapy products. Application of these strategies to cell measurements will help to establish qualified assays for cell characterization, which may help streamline regulatory approval and enable more efficient development of cell therapy products. *STEM CELLS TRANSLATIONAL MEDICINE 2016;5:705–708*

### SIGNIFICANCE

The regenerative medicine industry has identified the lack of reliable methods for measuring critical cell attributes as possibly the single greatest challenge facing the field. There are many strategies for achieving measurement assurance, or confidence in cell assays, which can streamline regulatory approval and enable more efficient development of cell-based therapies.

### INTRODUCTION

A recent survey conducted by the Alliance for Regenerative Medicine found that “product consistency and lack of standards is possibly the single greatest challenge facing the field” [1]. Similarly, a Regenerative Medicine Foundation survey concluded that the “lack of reference materials to benchmark measurements and validation criteria for critical assays” is the biggest roadblock for the field [2], and the National Cell Manufacturing Consortium identified the need for “product quality standards” as critical to the success of the industry [3]. At the root of these challenges is the need for quantitative, validated, and robust assays for characterizing cell therapy products (CTPs).

All cell therapy products, whether mesenchymal stem cells, T cells, or embryonic or induced pluripotent-derived cells, require reliable, robust measurements for product characteristics that support informed decision-making during their development, manufacturing, and regulation. A workshop, “Strategies to Achieve Measurement Assurance for Cell Therapy Products,” was held at the National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland, on May 11–12, 2015 [4], to convene industry, academia, regulators, and funding agencies (supplemental online Fig. 1a) and begin to examine approaches for improving confidence in the measurements that

are necessary for bringing CTPs to market (agenda is shown in supplemental online File 1 and breakout session templates in supplemental online File 2). The workshop focused on strategies for identifying, monitoring, and mitigating the sources of variability in the measurement process of cell assays. These strategies are the basis of what we refer to as measurement assurance. These measurement assurance approaches align with US Food and Drug Administration guidance on assay development [5], and an understanding of these principles can help to improve the quality and reliability of cell therapy product characterization.

### STANDARDS ARE UNDERPINNED BY MEASUREMENT ASSURANCE

There is general agreement that the development and regulatory approval process of CTPs can be accelerated by standardized measurement protocols for assays. Standard test methods will have the most benefit to the wider industry if they have broad application to many CTPs. In many cases, there is no need for wider standardization of product-specific methods that can be locally optimized and validated. This would hold true for a highly specific potency assay that applies to a single product. For broadly applicable methods that are candidates for wider standardization, the protocols must be amenable to

validation with sound measurement science. However, it is often unclear how to apply measurement science tools to cell assays, given the dynamic, complex, and heterogeneous nature of cells. Therefore, it may be premature to declare specific protocols as standards for the CTP field. In the absence of standardized protocols, measurement assurance strategies and tools can be applied to any measurement process to assess the confidence in the assay result. The successful use of measurement assurance strategies to establish highly qualified assays is the first step to realizing “standardized assays.”

#### TOOLS FOR ACHIEVING MEASUREMENT ASSURANCE

The concepts of measurement assurance have been applied in many chemical and physical measurements [6], and herein the application of these concepts to complex biological systems is discussed. Many analytical measurements are underpinned by stable reference materials (such as a known amount of lead in an aqueous solution), which provide a true value for an analyte. A laboratory can use that reference material to determine the amount of substance in their unknown sample. Such reference materials with a known value of an analyte are tools that provide a benchmark for an instrument reading, for determining limit of detection, or for an assay response function. Because the usefulness of a reference material is that it provides a result that is constant over time, inert materials are most often used.

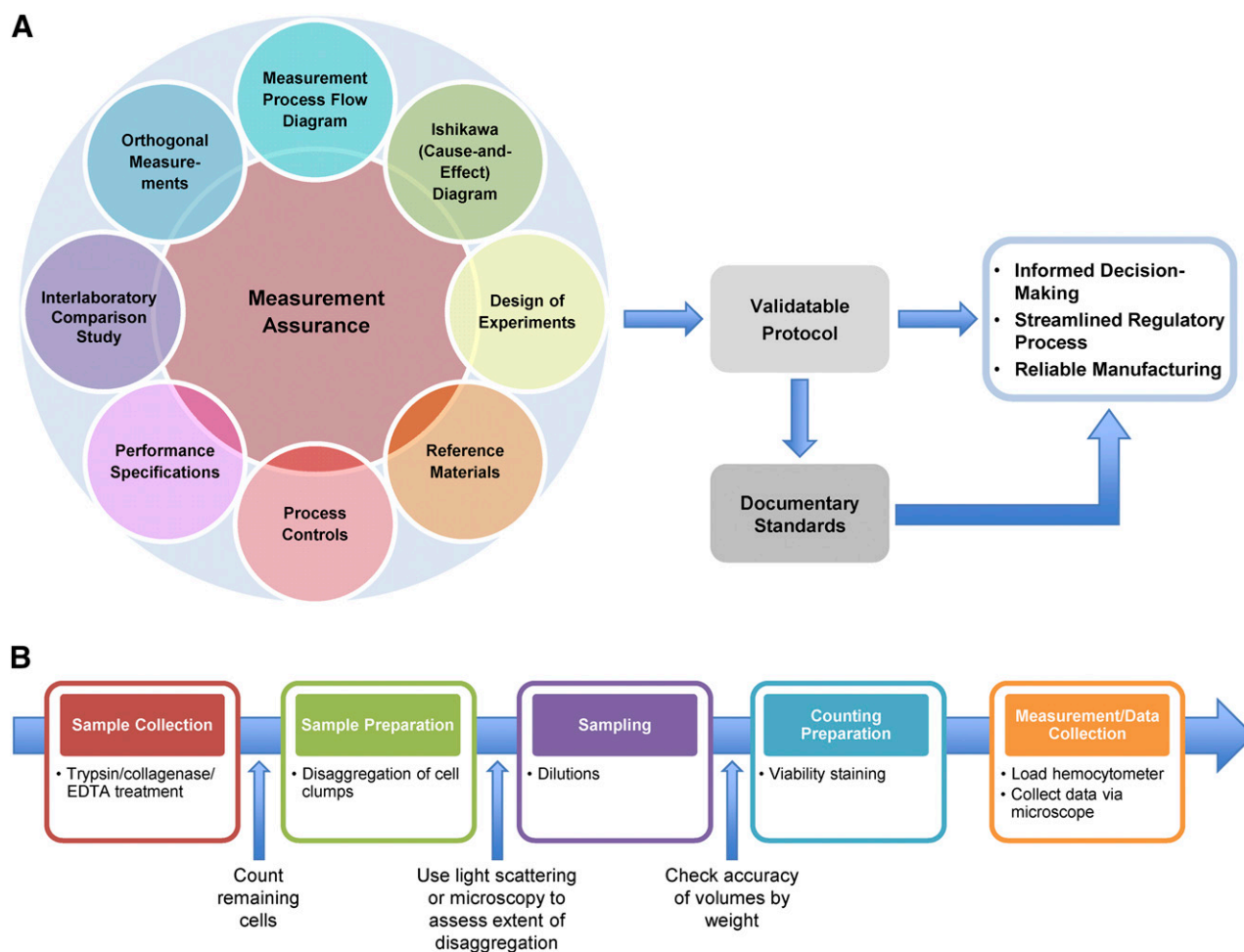
When cells are being considered as reference materials [7], the potential instability of living materials presents a challenge. By definition, living systems are capable of “adaption to the environment” [8], which is a disadvantageous property for a reference material that should have stable properties [9]. In addition, the lack of reliable cell measurements highlighted by the CTP industry [1–3] may complicate the process for establishing the reference values for the cell reference material. Cell preparations often serve as reference materials for in-house measurements, and cell lines have been proposed as reference materials to enable comparability of cell preparations [9] across laboratories. For academic laboratories engaged in cell therapy research, a cell reference material could be useful for serving as a reference point between different laboratories for comparing data. This would require that the cell reference material respond according to specifications under the diverse culture conditions in different laboratories and after shipping, storing, thawing, expansion, and passaging.

For clinical manufacturing, cell reference materials could be useful for reducing variability in the CTP characterization assays that use cells as part of the measurement system. An example discussed at the workshop is the tubulogenesis assay [10], in which the *in vitro* ability of primary vascular endothelial cells to form tubules when incubated in CTP-conditioned medium is measured as an indicator of the angiogenic potential of the CTP. The variability in the performance of the vascular endothelial cells in this assay impedes use of the tubulogenesis assay for CTP characterization. A cell reference material that reproducibly and reliably formed tubules may help reduce variability in this assay so that it could be useful for CTP characterization. Thus, development of a cell reference material could be advantageous for a number of uses, although careful consideration will be required to develop cell reference materials that are fit for purpose.

In the absence of reference materials, other strategies can be used to achieve measurement assurance in cell assays, such as recognizing and mitigating the sources of uncertainty, generating performance specifications through experimental process controls and other control experiments, and collecting objective evidence that adds to the confidence in the measurement process and the final assay result. These strategies provide a strong basis

for documentary standards and for decision-making (Fig. 1A). There is no one tool, method, or process for achieving measurement assurance; many approaches can be used. The following presents some of the tools for achieving measurement assurance.

- **Measurement process flow diagram:** By breaking down the measurement process into its component steps at an appropriate level of granularity, each step is identified and considered as a contributor to variability in the assay result. Ideally, this tool allows a developer to consider how step in a protocol contributes to assay variability and how controls applied at each step may be used to monitor and reduce variability in the assay result.
- **Ishikawa (cause-and-effect) diagram:** This graphical tool allows the identification of the parts of the process that are potential sources of variability. These sources of variability can influence several steps in a flow diagram (e.g., pipetting) or be specific to a particular step in an assay, such as the use of an instrument.
- **Design of experiments:** Not all of the likely sources of variability will contribute equally, and the relative effect of the various sources may be determined via a systematic design of experiments. Performing experiments in which assay parameters are varied can be called “sensitivity testing” or “ruggedness testing” because the goal is to identify sensitive assay parameters that must be controlled to make the assay more reproducible under different laboratory conditions.
- **Reference materials:** Reference materials enable traceability between measurements at different times and places. Reference materials can be of nonbiological or biological origin and can be used to calibrate or benchmark an instrument, or to provide an expected value in the measurement process.
- **Process controls:** Process controls are procedures to monitor critical control points in an assay and to check that assay steps are performing according to specifications. These control values should be documented and charted.
- **Performance specifications:** When an assay is well characterized, its characteristics, such as the process control results, the standard deviation between replicates, the lower limit of detection, and range of linearity of response, must be within a user-defined range of values to be acceptable. A suitable acceptance range (e.g., performance specifications) is established by tracking assay performance over time (charting). Documenting that specifications are met each time an assay is performed is evidence for validation. If the assay characteristics are outside of the specifications, the test result may not be valid for use in decision-making (e.g., lot release).
- **Interlaboratory comparison study:** Sources of variability may be difficult to recognize when a procedure is followed rigorously by a small number of individuals in a confined environment. One way to determine the robustness of an assay protocol to unexpected changes in assay conditions is through interlaboratory comparison studies. These studies can be designed to test various aspects of the measurement process, such as incubation times, manual agitation, cell culture procedures, reagent source, or storage conditions, by having different laboratories perform the same measurement. The data from an interlaboratory comparison study can result in refinement of an assay protocol to account for newly recognized sources of uncertainty, inclusion of a reference material that provides traceability between laboratories, or establishment of performance specifications. Interlaboratory studies are typically used to assess the reproducibility of a protocol across laboratories during the development of documentary standard test methods. CTP companies may use interlaboratory



**Figure 1.** Many tools are available for achieving measurement assurance. **(A):** Measurement assurance is achieved by identifying and mitigating the sources of uncertainty in the measurement process. This enables informed decision-making and underpins the development of documentary standards. **(B):** Flow diagram of generic and specific steps in a cell counting assay. Examples of process controls are shown between several steps. Others can be added.

comparability studies when trying to achieve reproducibility across multiple manufacturing/analytical sites.

- Orthogonal measurements: When two or more different measurement methods are used to make a measurement of the same sample and the results agree, this provides confidence in the accuracy of each method.

#### APPLYING MEASUREMENT ASSURANCE TOOLS

Cell counting is the most fundamental cell measurement, and all cell therapy manufacturers conduct cell counting measurements. Furthermore, cell counting/viability was the cell measurement that was the most frequently identified as “most in need of improvement” by an informal survey of 23 key stakeholders (supplemental online Fig. 1b). An example of how to use a flow diagram for a cell counting and/or viability assay is shown in Figure 1B. The assay is depicted as a series of discrete steps in a measurement process. Each step has the potential to be a source of variability. At each step, process control measurements can be used to assess the operation of that step. For example, a “sample collection” step could include processes such as trypsinization, collection from microcarriers, sampling from a bioreactor, collection from cryo-storage, or clinical sample collection. If a method for cell counting involves removing cells from a substrate,

the details of the process for carrying out this step will likely affect the efficiency of cell removal, which can influence the cell number, and therefore can be a significant source of variability. Figure 1B indicates that an intermediate metric for this step could be an evaluation of how many cells remain attached to the substrate after trypsin treatment.

The process of disaggregation of cell aggregates during a “sample preparation” step can also strongly affect the accuracy and reproducibility of the measurement process; poor disaggregation might result in undercounting, and too vigorous a disaggregation step can damage cell integrity. If the cell counting assay is coupled with cell viability, then this step is likely to be a highly significant source of variability in the assay result. For example, a control experiment could determine the relationship between the disaggregation procedure and the number of viable cells counted. Light scattering or microscopy could be used to determine the range of sizes of cell aggregates in the solution under conditions where the maximum number of viable cells was present. This measurement could be applied as a process control to provide assurance that the disaggregation step was executed as expected.

A “sampling” step might include dilution or concentration of the sample. A process control for preparing dilutions could involve weighing the aliquots to provide assurance of the accuracy and precision of that pipetting step. This would allow assessment

of the contribution of the variability in the dilution procedure to the total variability in the assay.

Although control experiments such as these are probably used in most laboratory settings, formalizing the process acknowledges the need for dedicating time and resources for developing an assay protocol and helps to ensure that significant sources of variability will not be overlooked. Control measurements such as these are useful for developing a new assay or examining a unique cell sample, but practical considerations will determine how often they will be applied. If an assay is particularly critical and is highly sensitive to a particular step in the protocol, then using a process control measurement for that step every time the assay is run may be advised. Reporting ancillary experimental data and the use of reference materials and process controls in addition to the assay results enable evaluation of the confidence in the result of the assay. These data also provide confidence to others (such as regulators or investors) that the measurement process is well controlled.

The potential sources of variability for the cell counting process can also be organized in an Ishikawa (cause-and-effect) diagram (supplemental online Fig. 2), which provides a graphical method to examine the factors that are major sources of variability and how they are related to one another. Applying a formal process for assessing sources of variability in an assay can help to reveal weakness in protocols. For example, an assay such as the tubulogenesis assay for testing in vitro angiogenic activity entails culturing vascular endothelial cells on an extracellular matrix and assessing the formation of tubules in response to the conditioned culture medium from the CTP [10]. One step in the process involves preparation of extracellular matrix, another step involves culturing endothelial cells on the matrix, a third step is treatment with conditioned medium, and a fourth step is assessment of tubulogenesis. The source of extracellular matrix material is challenged with inconsistent composition and activity. The sourcing of the endothelial cells is problematic because of variability in the tubule-forming activity of different cell preparations. The scoring of tubule formation is a manual process that is inconsistent because of operator bias and variability. At each step, there are significant challenges to controlling the sources of variability and achieving adequate control over the measurement process.

## FUTURE DIRECTIONS

Additional discussions within the cell therapy development community could help to develop measurement assurance strategies and protocols for specific assays that can be validated. A potential role for NIST could be to help coordinate these discussions. Cell counting and cell viability, which are fundamental to characterizing almost any CTP, may be appropriate topics for broad collaboration in precompetitive space. Standards organizations, such as ISO Technical Committee 276 Biotechnology and ASTM International Committee F04 Tissue Engineered Medical Products, are potential partners for converting these community-developed protocols into documentary standards for CTPs.

## ACKNOWLEDGMENTS

We are grateful to our steering committee for helping us to plan the workshop: Fouad Atouf (U.S. Pharmacopeial Convention), Kyle Kolaja (Cellular Dynamics International), Michael McCaman (Lonza), Steven Oh (U.S. Food and Drug Administration), Darwin Prockop (Texas A&M), Pamela Robey (National Institutes of Health), and Jon Rowley (RoosterBio). We also thank the speakers, breakout session organizers, and panel members for their thoughtful insight: Eytan Abraham (Lonza), Jessica Carmen (Alliance for Regenerative Medicine), Janet Davis (Janssen Research and Development), Michael Halter (NIST), Sandro Matosevic (Akron Biotech), Michael Mendicino (Mesoblast), Sagi Nahum (Pluristem), Yael Porat (BioGenCell), Mahendra Rao (Q-Therapeutics), Ivan Rich (Hemogenix), Marc Salit (NIST), Christopher Wiwi (CelGene), Juliana Woda (Juvantas Therapeutics), and Claudia Zylberberg (Akron Biotech).

## AUTHOR CONTRIBUTIONS

C.G.S., S.L.-G., J.T.E., S.S., and A.L.P.: conception and design, manuscript writing.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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