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Testing Strategies and Custom Assay Development for Cell and Cell-based Therapies

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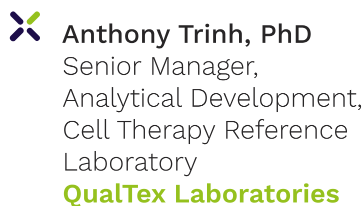
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Cell and cell-based therapies are a novel and growing class of transformative therapies designed to address gaps in traditional treatment strategies. Regulatory approval for cell or cell-based products requires therapeutic developers to have a set of analytical tests to demonstrate safety, purity and potency. These tests must be validated and performed under current good manufacturing practice (cGMP) conditions.

The number of cell and cell-based therapies are increasing at an exponential rate causing a capacity constraint for routine product testing and analytical assay development. This constraint has caused delays in product development, product supply, clinical trials, regulatory filings and approvals. Increased analytical development and testing capacity is critical to relieve this barrier in getting cellular therapies to market.
Pre-existing assays can be utilised for some of this testing, but some degree of custom assay development is usually required, especially regarding potency testing. QualTex Laboratories uses several testing strategies to help customers in the cell and cell-based therapy industry. These strategies include:

- Assay development process
- Criticality of potency assays
- Qualitative versus quantitative assays
- Use of blood screening safety assays
- Phase appropriate validations

Assay development process

Development of custom analytical tests involves a collaboration between therapeutic developers and the assay development team. Our process developing custom analytical tests for demonstrating safety, purity and potency of cell and cell-based therapies can be divided into six key stages:

1. Specifications

- First determine the exact goal and regulatory requirements for the assay
- Determine the volume of testing that will be performed, acceptable cost, range and expected turnaround-time for the assay

2. Design

- Establish an assay profile and feasibility criteria as early as possible in the development process
- Examine scientific considerations such as mechanism of action and relevant biological functions of the cell or cell-based therapy
- Determine appropriate platform, level of automation and if pre-existing assays can be modified to meet the clients need

3. Optimise performance

- Optimise each independent step with respect to the overall process
- Utilise statistics and international standards to guide optimisation

4. Validation

- Validate reagents, equipment and process using a GMP-compliant facility
- Utilise appropriate regulatory standards
- Use acceptable tolerance levels for assay sensitivity, specificity and reproducibility

5. Implementation

- Implement only after validation is completed, appropriate SOPs are in place and testing personnel have documented training

6. Long-term program management of the assay

- Monitor assay performance using key quality indicators and implement assay enhancements as needed

Criticality of potency assays

Determination of potency using specific assays is essential to transition cell and cell-based therapy products through clinical trials to final regulatory authorisation and commercialisation. Potency is defined as 'the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result'[1]. A therapeutic product cannot be licensed without validated potency assays regardless of clinical benefit[1]. Potency assessment provides reassurance that the cGMP manufacturing process is performing reliably to produce a consistent effective product. *In vivo* models used during proof of concept and efficacy studies provide some information on potency based on responses in animals. However, licensed product approval requires the development of a quantitative biological assay. If development of a quantitative bioassay is not feasible, a surrogate measurement of biological activity can be used but the surrogate measurements must be correlated to a relevant product-specific biological activity[1].

An example of a potency assay developed at QualTex Laboratories is an assay that measures the inhibition of peripheral blood mononuclear cells (PBMCs) in response to mesenchymal stromal cells (MSCs). MSCs have been shown to have immunomodulatory/anti-inflammatory activity on peripheral blood mononuclear cells. In the human body, peripheral blood mononuclear cells mount an immune response to anything foreign to the host such as allergens, microbial infection, donated-organs, foreign tissue or other stimulants. The immune response to such stimulants can be inferred by the increasing number of PBMCs in the blood. Since MSCs are immunomodulatory, the host should not recognise them as foreign and the PBMCs should not expand in the presence of potent MSCs. The immunomodulatory property of MSCs should mitigate the reactive expansion of PBMCs, despite the presence of a stimulant. This scenario can be captured as an *in vitro* potency test for MSCs (Figure 1). The presence of the MSCs inhibits the expansion of PBMCs.

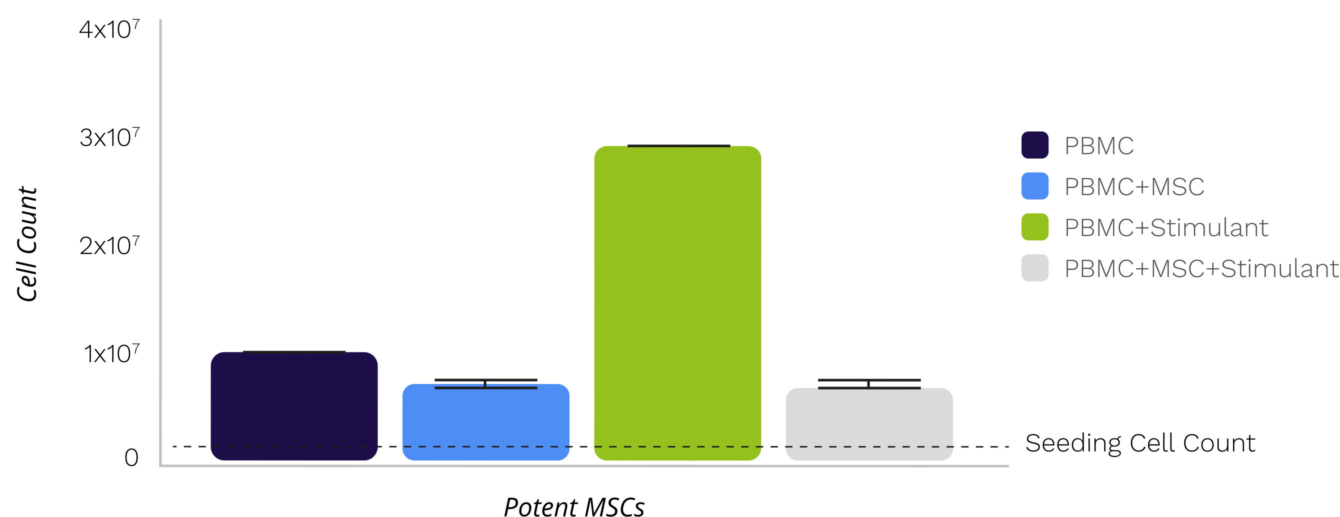

Figure 1. Inhibition of PBMCs in response to MSCs

1 U.S. FDA Guidance for Industry – Potency Tests for Cellular and Gene Therapy Products, CBER January 2011.

Quantitative vs qualitative assays

A critical quality attribute (CQA) is a physical, chemical, biological, microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the quality of the product[2]. These CQAs collectively define the safety, purity and potency of the biological product. The traditional approach for safety assays used to detect the presence of infectious agents in cellular based therapeutics have been quantitative PCR-based assays. There is currently no regulatory requirement that these safety assays be quantitative.

Typically, the limit of detection (LOD), a qualitative measurement of an analytical assay will be at a lower concentration than the limit of quantitation (LOQ), a quantitative measurement (Figure 2)[3] but how much lower depends on the specifications for bias and imprecision used to define it. We determined the LOD and LOQ values for commercially available EBV, CMV, Parvovirus B19, SV40 and HTLV-1/2 PCR assays used for safety testing of cell and cell-based therapeutics. A probit analysis was performed to determine the LOD of the Altona RealStar® EBV, CMV and Parvovirus B19 PCR assays and Genesig SV40, HTLV-1 and HTLV-2 PCR assays in bone marrow derived mesenchymal stromal cells (BM-MSCs).

Probit samples were tested a minimal of four times each for five days. Probit analysis was performed on a minimum of 20 replicates per viral concentration using Minitab. Linearity of each assay was determined using viral standards. The lowest value of the linear range was determined to be the LOQ for each assay. The LOD for all assays was lower than the LOQ. The LOD was 24, 1.9, 1.6, 2.8, 1.7 and 5.8 times lower for EBV, CMV, Parvovirus B19, SV40, HTLV-1 and HTLV-2 respectively (Table 1).

This data supports the use of qualitative PCR assays to detect the presence of infectious agents in BM-MSCs and provides evidence that the qualitative assay allows for higher sensitivity in detection of all viruses tested in our panel compared to the same assay performed in a quantitative manner. These qualitative assays are usually less expensive since samples tested qualitatively are usually performed once versus running replicates for quantitative testing.

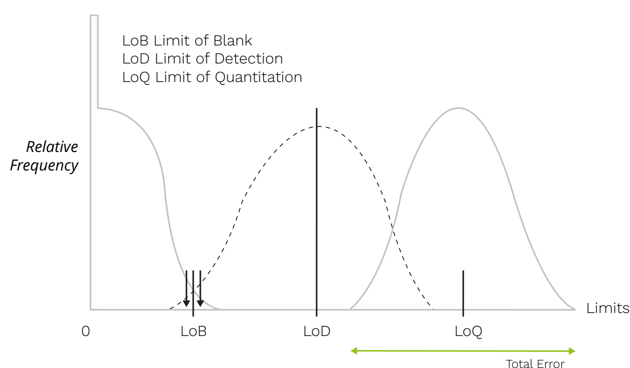

Figure 2. LOD and LOQ

Virus	LOD	LOQ
EBV	129.5 IU/mL	3162.2 IU/mL
CMV	159.6 IU/mL	316.2 IU/mL
B19	59.2 IU/mL	100 IU/mL
SV40	111.5 copies/mL	316.2 copies/mL
HTLV-1	57.3 copies/mL	100 copies/mL
HTLV-2	54.8 copies/mL	316.2 copies/mL

Table 1. Calculated LOD & LOQ values

2 International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use considerations (ICH) guideline Q8 (R2) on pharmaceutical development, June 2017.

3 Armbruster DA, Pry T. Limit of Blank, Limit of Detection and Limit of Quantitation. Clin Biochem Rev Vol 29 Suppl (i). 2008: S49-52

Use of blood screening safety assays

We validated automated high-throughput qualitative blood screening NAT assays to screen human bone marrow derived mesenchymal stromal cells (BM-MSCs) for HIV, HCV and HBV as an alternative to traditional diagnostic quantitative assays.

A probit analysis was performed to determine the limit of detection (LOD) of the Grifols Procleix® Ultrio Elite assay to detect HIV-1, HIV-2, HBV and HCV in BM-MSC samples. Probit samples were tested a minimal of four times each for five days. Probit analysis was performed on a minimum of 20 replicates per viral concentration using Minitab. Qualitative accuracy of the assays was measured by analysing viral concentration samples that were close to but above 3 x LOD as determined by the probit analysis. Specificity of the assays was also determined using BM-MSC samples spiked with international standard viruses. Intermediate precision and precision within runs were calculated on each day tested. Robustness of the assays was determined testing 20 replicates of BM-MSCs spiked at 3 X 95% Probit LOD using two different Grifols Hologic Panther® instruments. We also determined the highest cell concentration where HIV, HCV and HBV were detected.

The 95% LOD values for the different assays are shown in Table 2. All viral concentrations tested had a %CV ≤ 30% for intermediate precision and a %CV ≤ 20% for precision within a run. All samples tested during the robustness study were positive for the appropriate viruses, we did not see any inhibition of the assays by the BM-MSC samples. The highest concentration where HIV, HBV and HCV were consistently detected was determined to be 1 X 10^5 cells/mL.

We were able to demonstrate that high-throughput qualitative blood screening NAT assays originally designed for the screening of human serum and plasma samples displayed acceptable performance in LOD, specificity, precision and robustness to be used to screen human BM-MSCs for HIV, HCV and HBV. Since these are automated high-throughput assays it allows for faster and less expensive screening of cell and cell-based therapies for infectious agents.

Assay	95% LOD (IU/mL)
Procleix® Ultrio Elite HIV-1	96.5
Procleix® Ultrio Elite HIV-2	52.0
Procleix® Ultrio Elite HBV	4.2
Procleix® Ultrio Elite HCV	29.3
Procleix® Ultrio Elite dHIV-1*	60.0
Procleix® Ultrio Elite dHIV-2*	26.6
Procleix® Ultrio Elite dHBV	6.2
Procleix® Ultrio Elite dHCV*	16.5

Table 2. 95% LOD Values

*Discriminatory assays since the Procleix® Ultrio Elite assay is a multiplex (HIV-1, HIV-2, HBV, HCV) and is approved by the FDA for whole blood and plasma screening

Phase appropriate validations

For early process development, assays and analytical procedures may not need to be validated since many of the properties being measured may not be critical quality indicators. Early on analytical development of methods should support product safety and potency. Many times, methods, platforms and markers used to characterise a therapeutic will evolve during the clinical trial phase. Therefore, in the beginning the analytical analysis must stay flexible and adaptable. Methods should continually be monitored for suitability and if additional assay optimisation is needed.

One thing that must remain constant is any analytical procedure must be shown to be fit for its intended purpose before use. Moreover, the analytical method must have the basics of a SOP, instrument/software validation to ensure measurements are accurate, all analysts are properly trained, proper documentation of test results is maintained and data integrity is preserved.

During early process development, many attributes are assessed to determine the subset of CQAs to focus on during later stages of process development. These early assays are performed as research use only tests, meaning they are not fully validated only the CQAs require full cGMP validation using appropriate controls and standards. Newly developed assays should be evaluated to determine the specificity, linearity, range, accuracy, precision, LOD and reproducibility. Generally, acceptance criteria are wide and based upon developmental data.

In later phases, analytical methods should support monitoring of manufacturing consistency of a therapeutic. Late phase validation should again be evaluated for specificity, linearity, range, accuracy, precision (repeatability, intermediate precision and reproducibility), quantitation limit, LOD and robustness. Generally, acceptance criteria are narrower and based upon historical data.

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