Qualifying Blood Screening NAT Assays for Safety Testing of Cellular Based Therapeutics

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Background/Case Studies:

- A critical quality attribute (CQA) is a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the quality of the product.
- These CQAs collectively define the safety, purity and potency of the biological product. One CQA related to the safety of cellular-based therapeutics is testing for infectious agents.
- The traditional approach for safety testing is to use quantitative PCR-based diagnostic assays.
- The aim was to validate 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 assays.

Study/Design Methods:

- A Probit analysis was performed to determine the limit of detection (LOD) of the Panther Procleix Ultrio Elite assay to detect HIV-1, HIV-2, HBV and HCV in BM-MSC samples.
- Probit samples were tested a minimum 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 analyzing viral concentration samples 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 by testing 20 replicates of BM-MSCs spiked at 3 X 95% Probit LOD using two different Panther instruments.
- We also determined the highest cell concentration where HIV, HCV, and HBV were detected.

Results/Findings:

Table 1: 95% LOD Values

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

- 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.

Conclusion:

- 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 potentially less expensive screening of cell and cell-based therapies for infectious agents.