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Recommendations for Demonstrating Equivalency of Ready to Assay Cells in Neutralizing Antibody (NAb) Methods Post-Validation

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Abstract

Cell-Based Neutralizing Antibody Methods rely on meticulous reagent management programs to ensure robust and sensitive screening methods. The use of single use, thaw and plate cell reagents is rapidly gaining credibility as a suitable alternative to continuous culture. Single use cell reagents offer improved precision and robustness paired with ease of use. In cases where a method has been previously validated with continuous culture, it is necessary to ensure the screening cut point and sensitivity are not impacted by a new critical cell reagent. This case study demonstrates the measures to apply in confirming single use cell vial performance, thus minimizing risk to clinical programs

Method: A human erythroleukemic cell line (TF-1) was cultured and expanded for creation of (2) single use cell banks at target density. Cell yield and viability were assessed with acceptance criteria as defined in the validated method. Cells were plated (1 vial per plate) to demonstrate inter-vial seeding variability. Human sera samples and reference neutralizing antibody controls were incubated with therapeutic for 1 h prior to treating cells. Cells were incubated 72 before application of Resazurin to measure cell proliferation.

Conclusion: Single use cell reagents may be used as qualified reagents in methods validated with continuous cell cultures. Recommendations include use of ≥ 4 cell vials with ≥ 2 analysts ≥ 2 days. The risk to immunogenicity screening should be managed with confirmation of validated method performance in required parameters: precision, robustness, sensitivity and concordance testing with validated cut point.

Purpose:

Regulatory agencies recommend the use of in vitro Neutralizing Antibody (NAb) Assays within Immunogenicity screening programs. Management of cell culture requires advanced training and preparation time to support cell-based NAb methods over the course of sample testing. Single use cell reagents offer improved precision and robustness paired with ease of use. In cases where single use Ready-To-Assay (RTA) models are not readily available prior to method validation, a qualification strategy may be employed to enable post-validation RTA reagent qualification.

Materials & Methods:

Human erythroleukemic cell line (TF-1 ras) was provided by Stemline Therapeutics. Cells were cultured and expanded for creation of (2) single use cell banks at target density. Human sera samples, including concordance samples from original validation were sourced from Bioreclamation.

Cell Yield and Viability were assessed through use of BD Biosciences Vi Cell Analyzer and Microscopy. Cell viability and density were compared to acceptance criteria as defined in the validated method. Cells were plated (1 vial per plate) to demonstrate inter-vial seeding variability.

Resazurin Viability/Proliferation: Positive control reference neutralizing antibody controls were prepared at 10 and 50 µg/mL. Samples were diluted 1:20 and incubated with therapeutic for 1 h prior to treating cells. Cells were incubated 68-72 with sample/therapeutic cocktail prior to application of Resazurin to measure cell proliferation on EnVision Multilabel Plate Reader at Ex544/Em590 nM.

Flow Cytometery: 500,000 cells/mL human TF-1 cells were treated for 72 h with therapeutic or media control. Cell pellets were resuspended in FACS buffer, then incubated with 7- AAD for 30 minutes. Following a wash and final centrifugation, the supernatant was removed and the cell pellet resuspended in PBS. Cells were subjected to analysis on a BD FACSCanto™ with analysis performed using FACSDiva™Software.

Sensitivity samples were prepared at the following concentrations: 0, 0.08, 0.23, 0.69, 2.1, 6.2, 18.5, 55.5, 166.6 and 500 µg/mL of neutralizing antibody.

Concordance Testing between continuous culture and RTA cells was performed using 30 healthy human serum lots used in validation. Sample responses represent mean of 2 assays with 3 analysts.

Therapeutic Mechanism of Action

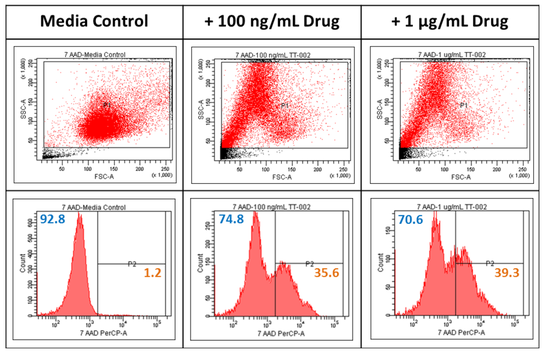

Figure 1: Flow Cytometry 7-AAD Staining for TF-1 ras Cell Viability.
Above Panel: Apoptotic cells demonstrate granularity (SSC) and DNA staining with 7-AAD upon treatment with drug (TT-002).
Below Panel: Percent distribution of Viable Cells (in blue) compared to Apoptotic Cells (orange).

Cell Viability at Method Seeding

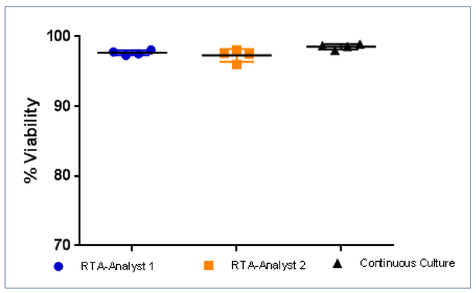

Figure 2: Cell viability was measured upon thawing of cryopreserved RTA vials prior to plating in method. Comparison of RTA vs Continuous Culture demonstrates equivalent cell viability as measured with ViCell Counter.

Cell Apoptosis in Continuous Culture vs RTA Model

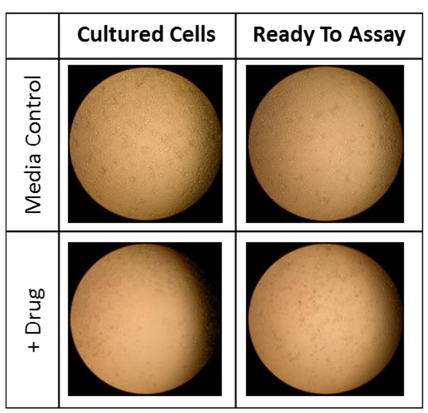

Figure 3: Viable density of continuous cultured cells vs RTA cells treated 72 h with media or therapeutic (40 ng/mL) demonstrates equivalence.

Cell Response and Signal Window

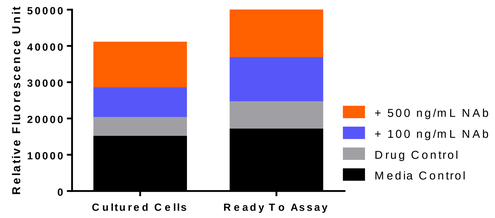

Figure 4: Comparison of Cell Model Signal Window and Sensitivity to Neutralizing Antibody. Signal window (Drug Control/Media Control) results are similar between cell models.

Neutralizing Antibody Sensitivity

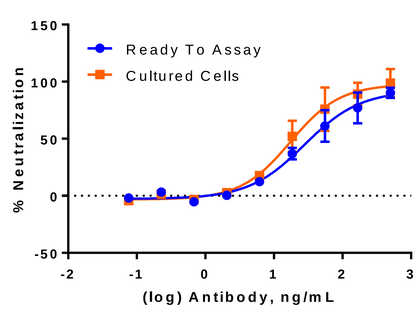

Figure 5: Positive Control neutralizing antibody sensitivity curve comparison or RTA and continuous culture. Sensitivity reports at 5.2 ug/mL (RTA) compared to 4.7 ug/mL (CC)

Positive Control Antibody Precision with RTA Model

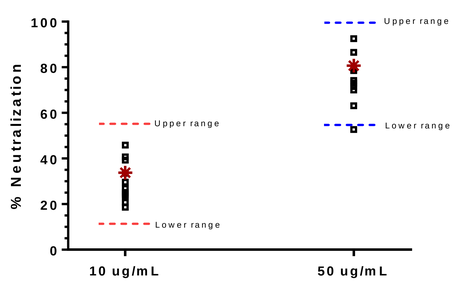

Figure 6: Positive Control neutralizing antibody (10 µg/mL and 50 µg/mL) performance with use of RTA reagents compared to Validated Precision Mean (*) and Range. For continuous culture cell line, the validated mean precision (% Neutralization) for 10 µg/mL and 50 µg/mL NAb were 33.8% and 80.7%, respectively. When tested with RTA cells, precision (% Neutralization) for 10 µg/mL and 50 µg/mL NAb were 29.9% (% Diff. of -11.7) and 74% (% Diff. of -8.3), respectively.

Concordance Testing of Cut Point Samples with RTA Model

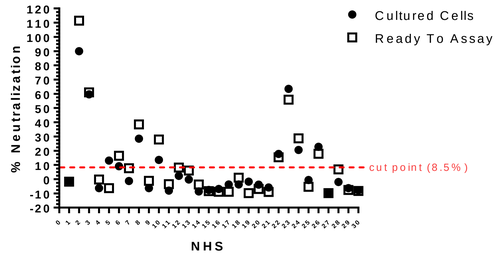

Figure 7: Concordance testing of validation cut point samples with RTA cells compared to validated fixed cut point. A combined 58 out of 60 results (29 out of 30 from each analyst) generated with RTA cells show concordance with original validation results for Normal Human Serum using continuous culture cell line.

Recommendations for RTA Cell Reagent Qualification

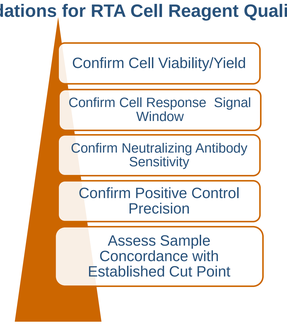

Figure 8: Recommended Strategy for Qualifying Ready to Assay (RTA) Cell Reagents Post-Validation

Summary

Ready to Assay cell formats may be subjected to post-validation qualification as critical reagents.

Conclusion

In selected cell models and methods, single use cell reagents (RTA cells) may be qualified as critical reagents in methods originally validated with continuous cell cultures. Qualification of single use cell batches should follow practices established for critical reagent management.

Recommendations include critical reagent qualification of ≥ 4 RTA vials with ≥ 2 analysts ≥ 2 days. RTA models should demonstrate equivalent cell viability/yield upon cell seeding to plate. Cell response signal window (S/B) and reference antibody neutralization should demonstrate results that meet method acceptance criteria. The risk to immunogenicity screening should be managed through documentation of equivalence to validation results: precision, sensitivity and concordance of validated screening cut point paired with robustness demonstrated through qualification assays performed over multiple days with multiple analysts

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