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R&D SYSTEMS

Application Note

Reliable Quantification of Cell Potency: Simple Plex Assays in Common Cell Media

According to the American Society of Gene & Cell Therapy, as of October 2023 nearly 2,300 ex vivo cell therapies are currently in development from pre-clinical to pre-registration[1]. These therapies have shown promise in treating many different cancers and other diseases. It is imperative that analytical cytokine release assays accurately measure the secreted proteins to demonstrate cell potency during therapy development and manufacturing[2].

Simple Plex™ assays on Ella™, an automated benchtop immunoassay platform, offers robust and reproducible data due to its hands-free workflow, high precision, and quick (<90 min.) time to results. Here, we evaluate the performance of various potency and characterization assays on Ella across commonly used immune cell culture media matrices and cryopreservation.

Robust Performance of Simple Plex Assays Across Common Culture Media

Differences in media formulation can potentially affect assay performance by impacting properties such as accuracy and dilutional linearity[3]. To evaluate the potential effects of media formulation on assay accuracy, we performed spike and recovery experiments on the most common cytokine release proteins (IFNg, TNFa, IL-2, IL-15, IL-17) and cytotoxicity proteins (Granzyme B and Perforin) (**Table 1A**). For each protein assay, matrices were spiked with known analyte concentrations at three levels (High, Medium, Low), and across seven commonly used and commercially available immune cell culture media (**Table 1B**).

TABLE 1. Reagents used in this study

A.

Reagent	Manufacturer	Catalog Number
Simple Plex Assays on Ella		
IFNg 3rd gen	R&D Systems	SPCKB-CS-002697
TNFa 2nd gen	R&D Systems	SPCKB-CS-002852
IL-2	R&D Systems	SPCKB-CS-000955
Granzyme B	R&D Systems	SPCKB-CS-000575
IL-15	R&D Systems	SPCKB-CS-003688
IL-17	R&D Systems	SPCKB-CS-003676
Perforin	R&D Systems	SPCKB-CS-007346

B.

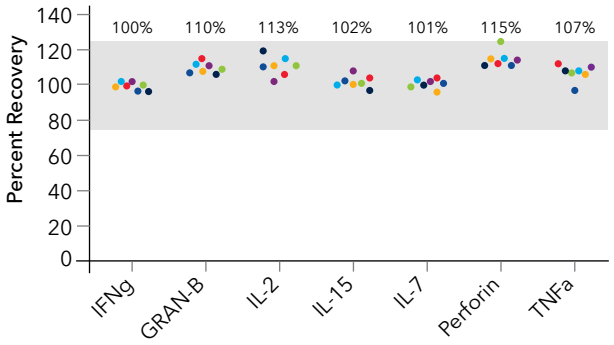
Reagent	Manufacturer	Catalog Number
Cell Culture Media		
ExCellerate (T Cell expansion)	R&D Systems	CCM030
OpTmizer CTS	Gibco	A10221-01
AIM V	Gibco	12055091
TexMACS	Miltenyi Biotec	130-097-196
PrimeXV T Cell expansion XSFM	IrvineScientific	91141
XVivo10	Lonza	04-380Q
LymphoOne	Takara	WK552S

The results support a high degree of accuracy across all assays and spiking levels tested (regardless of the cell culture medium used), with mean recovery rate of 100%-115% per assay, at a recovery range of 96-124% (**Figure 1**). Having demonstrated robust accuracy across media and assays, we next evaluated the dilutional properties of the assays under similar conditions. Importantly, we sought to verify that analyte concentration levels are reliable regardless of the amount of culture media present within the sample. Dilutional linearity experiments were carried out by performing serial dilution of spiked media, starting at the Minimum Required Dilution (MRD) of the assay. Notably, without exception, all assays and media tested yielded a high level of dilutional linearity, with mean linearity rate at 95-100% per assay, at a range of 92-108% (**Figure 2**).

Taken together, the spike/recovery and dilutional linearity results support the robustness and reliability of data obtained with Simple Plex assays for samples using these cell culture media. While each additional matrix is unique and may require separate evaluation, the fact that all commercial media tested in this study yielded equivalent and robust performance over multiple Ella assays suggests an overall high resilience of the assays to changes in media formulation.

FIGURE 1.

A. Spike/recovery across media



B. IFNg spike/recovery across media

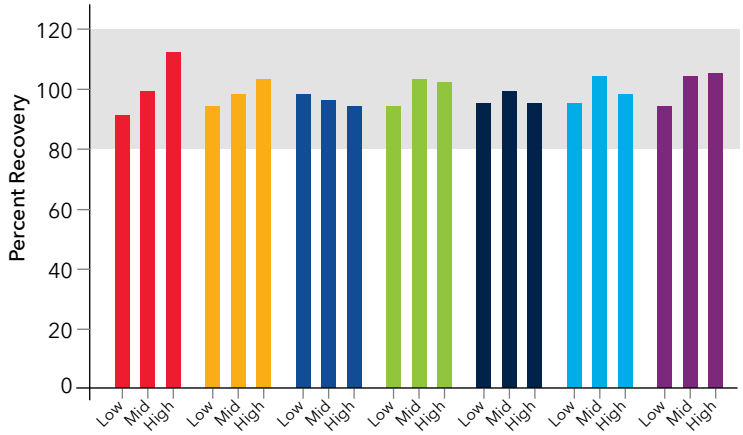
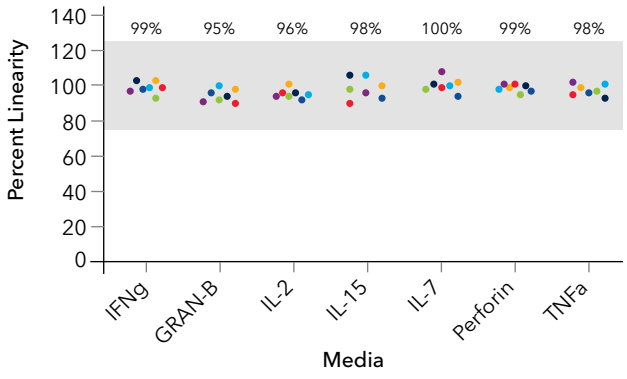


FIGURE 1. Seven cell culture media were spiked with high, mid, and low concentrations of recombinant protein controls in duplicates. Recovery percentages are shown and demonstrated good yield within assay specifications. A) All assays had mean recovery rates within 80-120% of expected for high, mid, and low spikes in all media tested. B) IFNg Simple Plex Assay demonstrates good recovery across high, mid and low spikes for all culture media.

Fig. 1 & 2 color key: ExCellerate · OpTmizer CTS · AIM V · TexMacs · PrimeXV · XVivo10 · LymphoOne

FIGURE 2.

A. Mean dilutional linearity across media tested



B. IFNg Simple Plex assay spiked dilutional linearity

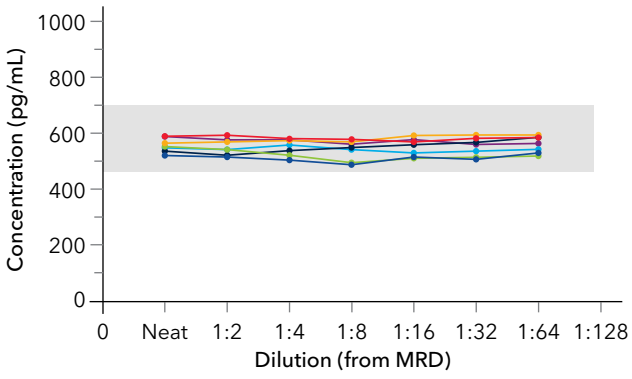


FIGURE 2. Cell culture media were spiked with recombinant protein and a serial dilution was performed from the assay MRD across the dynamic range of each assay. A) Example of IFNg Simple Plex assay shows consistent pg/mL concentrations of IFNg protein with serial dilutions in all media types. B) Mean dilutional linearity across media tested was found to be within expected range for all protein assays and media..

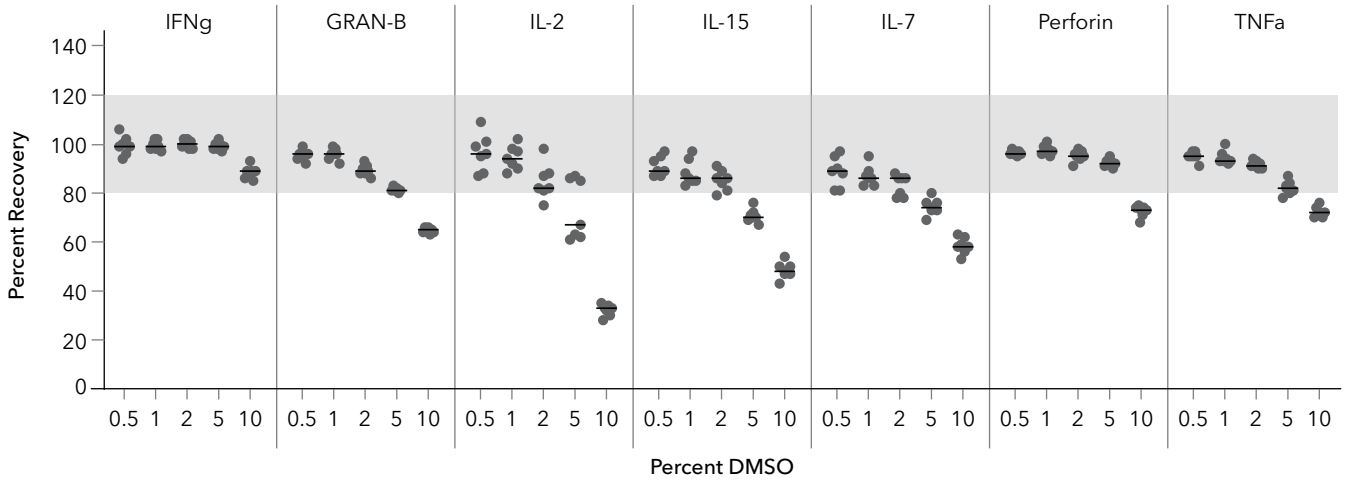
Assay Stability in the Presence of DMSO

Dimethyl Sulfoxide (DMSO) is a common organic solvent used for cryopreservation. Therefore, residual DMSO may be present in some cell therapy samples upon cell resuscitation. Because organic solvents can potentially affect protein properties and interactions, we evaluated DMSO tolerance across various Ella cell potency assays.

Utilizing the same assay and media panel that was used for accuracy and dilutional linearity evaluation (**Table 1**), spiked media samples were analyzed at DMSO concentrations ranging from 0% to 10%. As shown in **Figure 3A**, no significant interference was found in the presence of up to 1% DMSO within the cartridge well. This was demonstrated across all assays and media tested, yielding analyte concentration values within an acceptable range (defined as >80% recovery when compared to control samples that do not contain DMSO). Raising the DMSO levels above 2% revealed assay-specific effects. For example, while IFNg 3rd gen assay remained stable at DMSO levels up to 10% (**Figure 3B**), assays such as IL-2 and IL-15 exhibited suppression of signal at similar DMSO levels. Interestingly, the composition of the cell culture media did not play a key role in the sensitivity of the assay to DMSO, yielding similar DMSO sensitivity in a given assay regardless of the specific cell culture media used.

FIGURE 3.

A. Protein tolerance to dimethyl sulfoxide (DMSO)



B. IFNg Tolerance to dimethyl sulfoxide (DMSO)

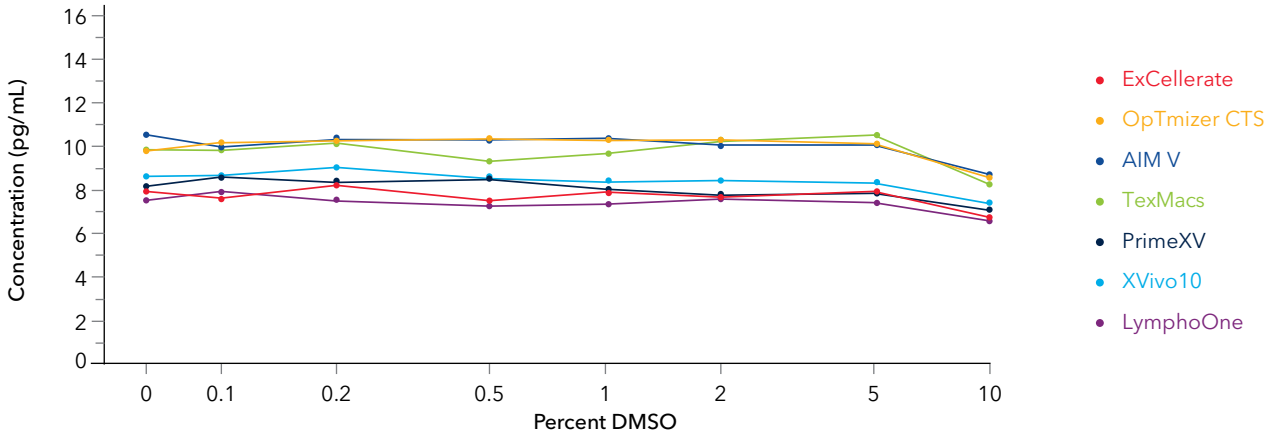


FIGURE 3. Cell culture media were spiked with a known concentration of recombinant protein and 0-10% DMSO. A) Summary of DMSO tolerance results across various Simple Plex assays. Each data point represents the normalized recovery of analyte levels as compared to its levels in the absence of DMSO. At final concentrations above 1% DMSO, the effects on the concentration values were assay specific. B) IFNg concentrations were largely unaffected by the presence of DMSO at concentrations of up to 10% across all media tested.

Conclusion

There are many variables that can impact assay performance. Understanding and addressing matrix effects is important in ensuring accurate data collection. In this work, we investigated the performance of various Ella potency and characterization assays in the context of immune cell culture work. Our results demonstrate robust performance of these assays across multiple, widely used immune cell culture media. Furthermore, tolerance studies support the resilience of these assays to a relatively wide range of residual organic solvent concentrations. Thus, the Simple Plex/Ella technology offers unique biomarker analytical solutions within the cell therapy workflow that remain robust and effective across multiple media and conditions.

References

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2. Capelli, C., et al. (2023). Potency assays and biomarkers for cell-based advanced therapy medicinal products. *Frontiers in immunology*, 14, 1186224. https://doi.org/10.3389/fimmu.2023.1186224
3. U.S. Department of Health and Human Services. Food and Drug Administration. (2018). *Bioanalytical Method Validation Guidance for Industry*. Maryland: Office of Communications, Division of Drug Information Center for Drug Evaluation and Research.