ELISA, Luminex® Assays,
Simple Plex Assays on Ella

4 Essentials of Immunoassay Quality

Ensuring Robust, Reproducible, and Validated Results

bio-techne® | R&D SYSTEMS

Accurate and reliable immunoassay data is indispensable for healthcare

From clinical research and trials to critical quality attributes (CQAs) for bio-therapeutic manufacturing, immunoassays are an indispensable tool to reliably measure protein levels.

Immunoassay sensitivity and specificity over the long term are key for quantifying CQAs in simple matrices, as well as biomarkers in complex matrices such as serum and plasma.

The onus is on the immunoassay manufacturer to enable accurate and reliable data and minimize waste and unnecessary customer expense. Most importantly, measurements for functional activity and potency assays are necessary for patient efficacy and safety.

Our technical competency means the Immunoassay product families have expanded to next-generation ELISA-based platforms like multiplexing panel kits on Luminex® and Simple Plex assays on the automted ELISA system called Ella.

Explore | bio-techne.com/methods/immunoassays

R&D Systems is the **industry-leading developer and manufacturer** of antibodies, proteins, and ELISAs.

With over 40 years of experience and control of raw material supply, no other vendor can match R&D Systems' quality needed for clinical applications.

Under an ISO13485 facility, rest assured there are quality control processes within a robust quality system for accurate documentation, change controls and change notification to end-users .

By meeting the standards set by regulatory bodies, contract research organizations (CROs) and manufacturers can bring their therapeutic development to the market faster.

What are **matrix effects**?

A sample matrix is an accumulation of anything in your sample that can interfere or cross-react, affecting the ability to accurately quantify the target of interest. Some examples include protein binding partners, rheumatoid factors, anti-animal antibodies, albumins, pH, or salt concentration.

R&D Systems **Minimizes** Matrix Effects

Proteins

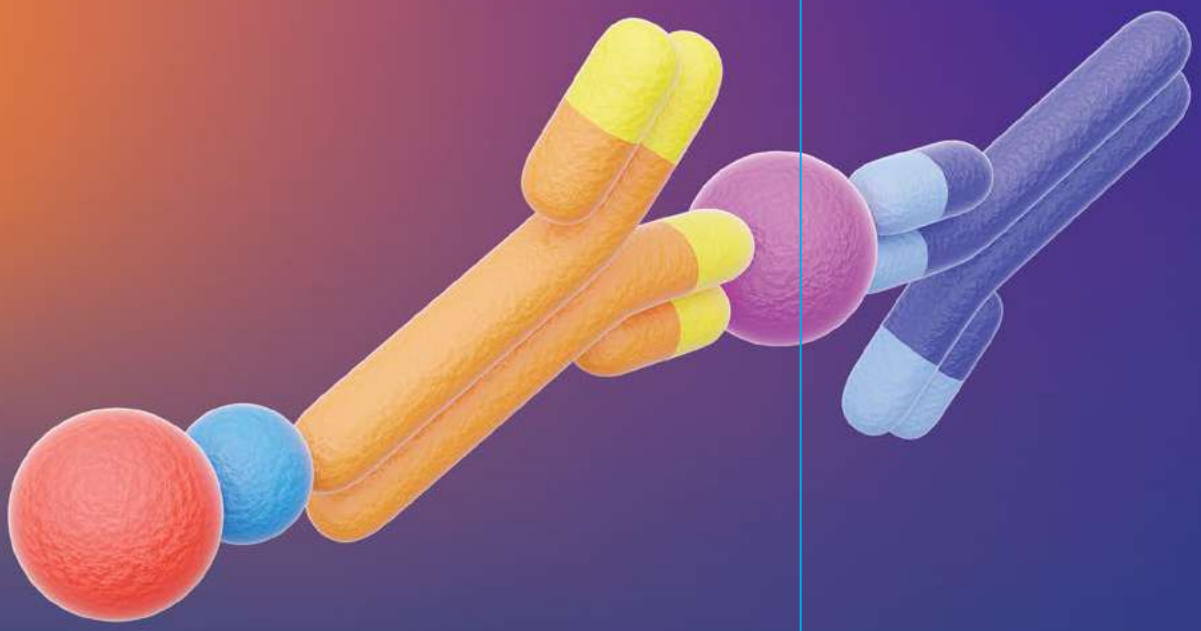
Highly purified in-house full-length recombinant proteins are used to calibrate assay standards. Natural matrix controls are used to calibrate standards for complex matrices such as human serum and plasma. As appropriate, recombinant proteins and natural matrix controls are used to establish master calibrators, which ensure the long term consistency of our immunoassays.

Antibodies

We carefully select our in-house antibodies to avoid cross-reactivity with substances in the sample. We exhaustively test our antibody pairs against related epitopes to ensure analyte specificity.

Diluents

We have a wide range of diluents that are optimized with blocking reagents to minimize non-specific binding and matrix effects. We formulate new diluents if interfering factors are found in our recovery tests.



4 Essentials of Immunoassay Quality

Quality immunoassays are necessary for patient safety

1
Recovery

2
Linearity

3
Specificity

4
Consistency

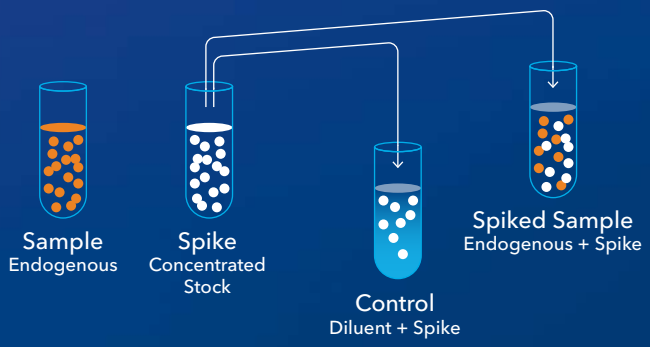
1

Recovery

Recovery, or spike recovery, is a measurement of accuracy.

A spike recovery study provides an estimate of how much of the available analyte is measurable in the assay and if there may be sample components interfering with that measurement.

R&D Systems assays are optimized for recovery at three different spike concentrations (low, mid, high) and for every validated sample type.



To perform spike recovery, a concentrated recombinant protein, typically the standard, is spiked into a complex sample matrix, such as serum or plasma. A control spike sample is the same amount of recombinant protein spiked into the diluent.

$$\text{Recovery Percentage} = \left(\frac{\text{Concentration}_{\text{Spiked Sample}} - \text{Concentration}_{\text{Sample Endogenous}}}{\text{Concentration}_{\text{Control}}} \right) \times 100$$

The readout of the spike into the complex sample, minus endogenous, should be equal to the control spike. 100% is ideal. If your sample under-recovers, or falls below 70-80%, it suggests that your quantitative results may be lower than the actual protein level.

The following data compares R&D Systems Luminex® assay and QuicKit ELISA recovery to leading competitors.

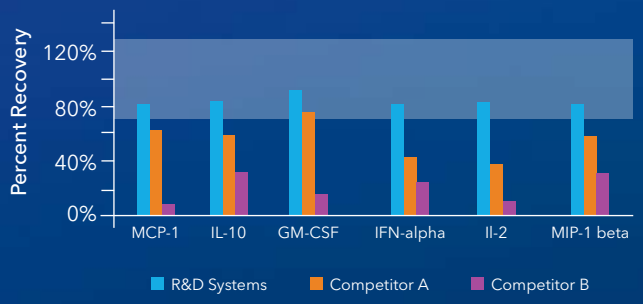


Figure 1. R&D Systems Luminex Assay Recovery Competitor Comparison. Recombinant proteins were spiked into human serum. The Human XL Cytokine Performance Luminex Panel (Catalog **#FCSTM18B**) outperformed other suppliers' assays when tested for recovery. Only the R&D Systems Luminex Assay consistently recovered 70-130% (grey bar). Both competitor assays consistently under-recover indicating that they are susceptible to matrix effects, even when using the same Luminex xMAP technology.

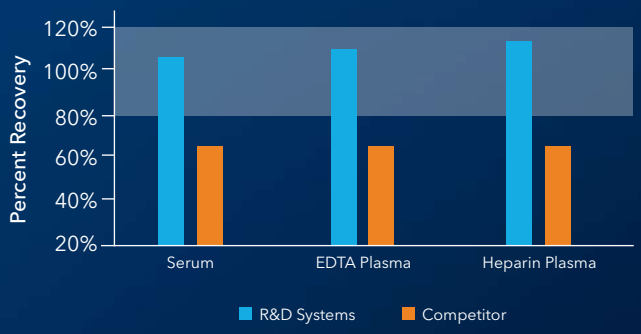


Figure 2. Quantikine QuicKit ELISA Recovery Competitor Comparison. Recombinant IL-1 beta was spiked into human serum, EDTA plasma, and Heparin plasma. The R&D Systems Human IL-1 beta Quantikine QuicKit ELISA (Catalog **#QK201**) average percent recovery is 80-120% (grey bars). The leading competitor rapid ELISA data is under-recovered indicating that it is susceptible to matrix effects.

Linearity

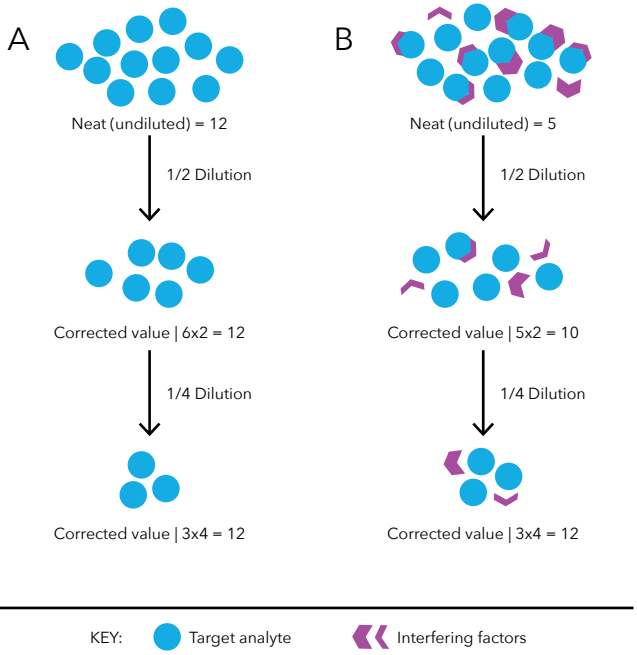
Linearity is a measure of accuracy with diluted samples.

When running diluted samples, you should derive the same concentration once back calculated. If the sample matrix is not properly controlled for, sample values often increase as you perform higher dilutions.

As you dilute the sample, the interfering factors also dilute out, making the target analyte more accessible, and the concentration of the target analyte increases (as depicted in the image on the right in Column B, where the corrected value should be equal to the neat value).

If endogenous levels are low, you can spike recombinant protein into your sample and dilute it out, which is commonly referred to as spiked linearity.

In R&D Systems assays, all sample types are optimized for linear dilutions through the standard curve range. Below, we demonstrate linearity of dilution using QuicKit ELISA and Simple Plex™ assays.



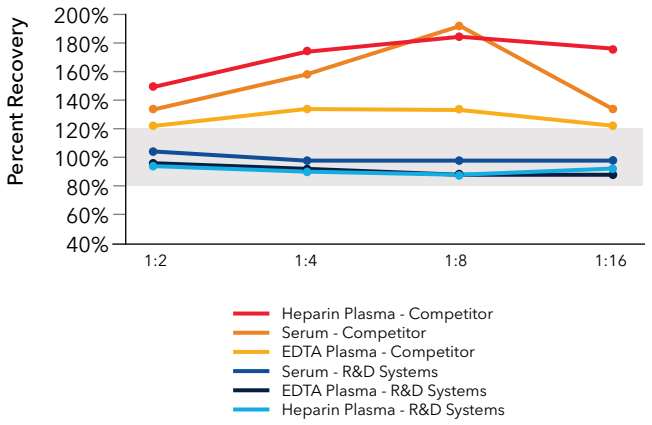


FIGURE 3. Human TNF-alpha QuicKit Spiked Linearity Competitor Comparison. TNF-alpha is spiked at high concentration in various sample matrices, then diluted with the appropriate calibrator diluent and run on the Human TNF-alpha Quantikine QuicKit ELISA (Catalog #QK210). R&D Systems ELISA meets the acceptable range (80%-120%, grey bar). The competitors over recovered, indicating susceptibility to matrix effects.

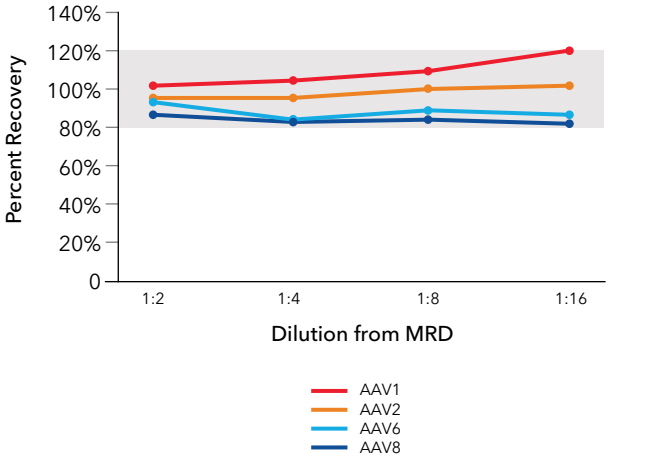


Figure 4. Simple Plex Assay Linearity of Dilution. Adeno-associated virus (AAV) linearity of dilution was evaluated using in-process samples across multiple sample matrices. Linearity of dilution was tested using AAV1 (Catalog #SPCKB-OT-007969), AAV2 (Catalog #SPCKB-OT-005905), AAV6 (Catalog #SPCKB-OT-007971), and AAV8 (Catalog #SPCKB-OT-007970) in-process samples. Each in-process sample showed good dilutional linearity at all four dilutions tested, with 80-120% percent recoveries compared to the minimum required dilution (MRD).

Specificity

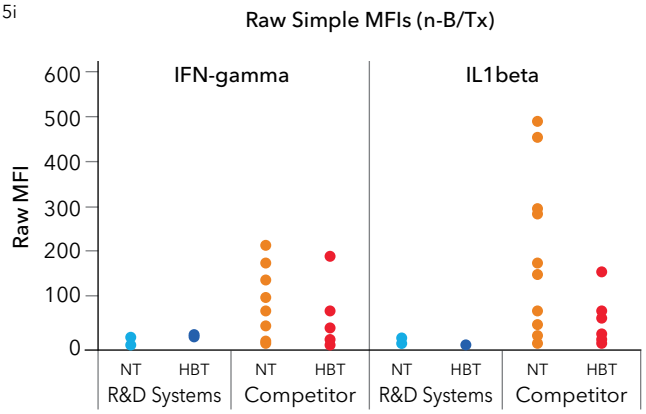
3

Specificity, or true negative rate, is confidence in a negative test result.

Immunoassays are subject to several potential interferences from components in complex samples like serum or plasma. The nature of these interferences can be both positive and negative as shown below. All result in an inaccurate value.

One of the most important features of an immunoassay is that you are measuring the correct intended analyte, so you can have confidence in results and move your research forward.

5i



5ii

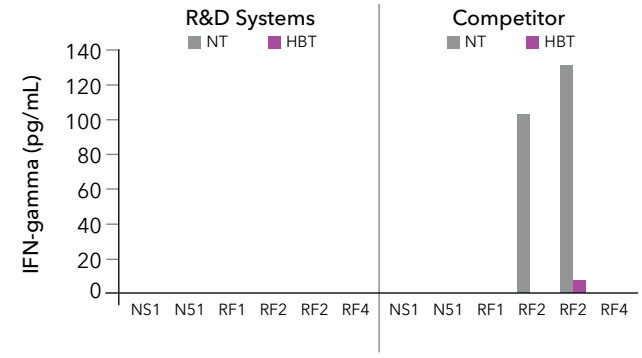
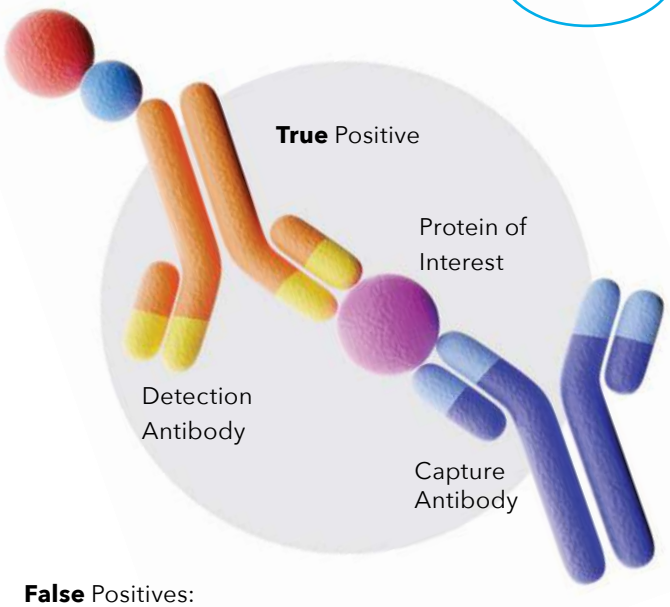
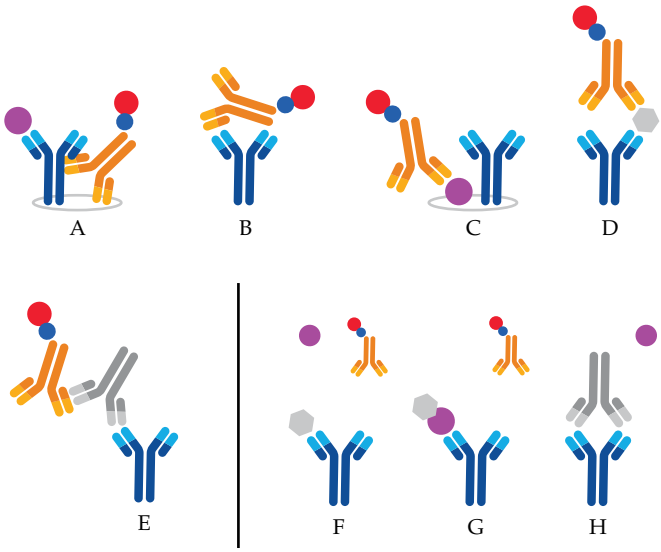


Figure 5. Removal of False Positives. Samples from patients with known values of rheumatoid factor (RF) or HAMA, both of which are known to be potential interferants in immunoassays. Subsets were treated with a commercial quenching agent (HBT) designed to block those interferences and the other subset was left untreated (NT). All samples were run in both R&D Systems and in a competitor's Luminex assay. Chart 5i, top most, is a composite graph showing all samples for IFNg and IL-1b. The competitor's assay is prone to false positive interference while the R&D Systems assay is not. The bars in Chart 5ii, immediately above, represent false positive values in the competitor's assay, and no false positives in the R&D Systems assay.



False Positives:

A. Non-specific binding of detection antibody
B. Capture antibody binds to the Fc segments of detection antibody
C. Non-specific binding of analyte to plate
D. Cross-reactivity of both antibodies to similar protein
E. Bridge binding back heterophilia antibodies or AMAS



False Negatives:

F. Interference by something bound to capture antibody
G. Interference by something bound to analyte
H. HAMA (human anti-mouse antibodies) interferes with the capture antibody

3

Specificity

Specificity, or true negative rate, is confidence in a negative test result.

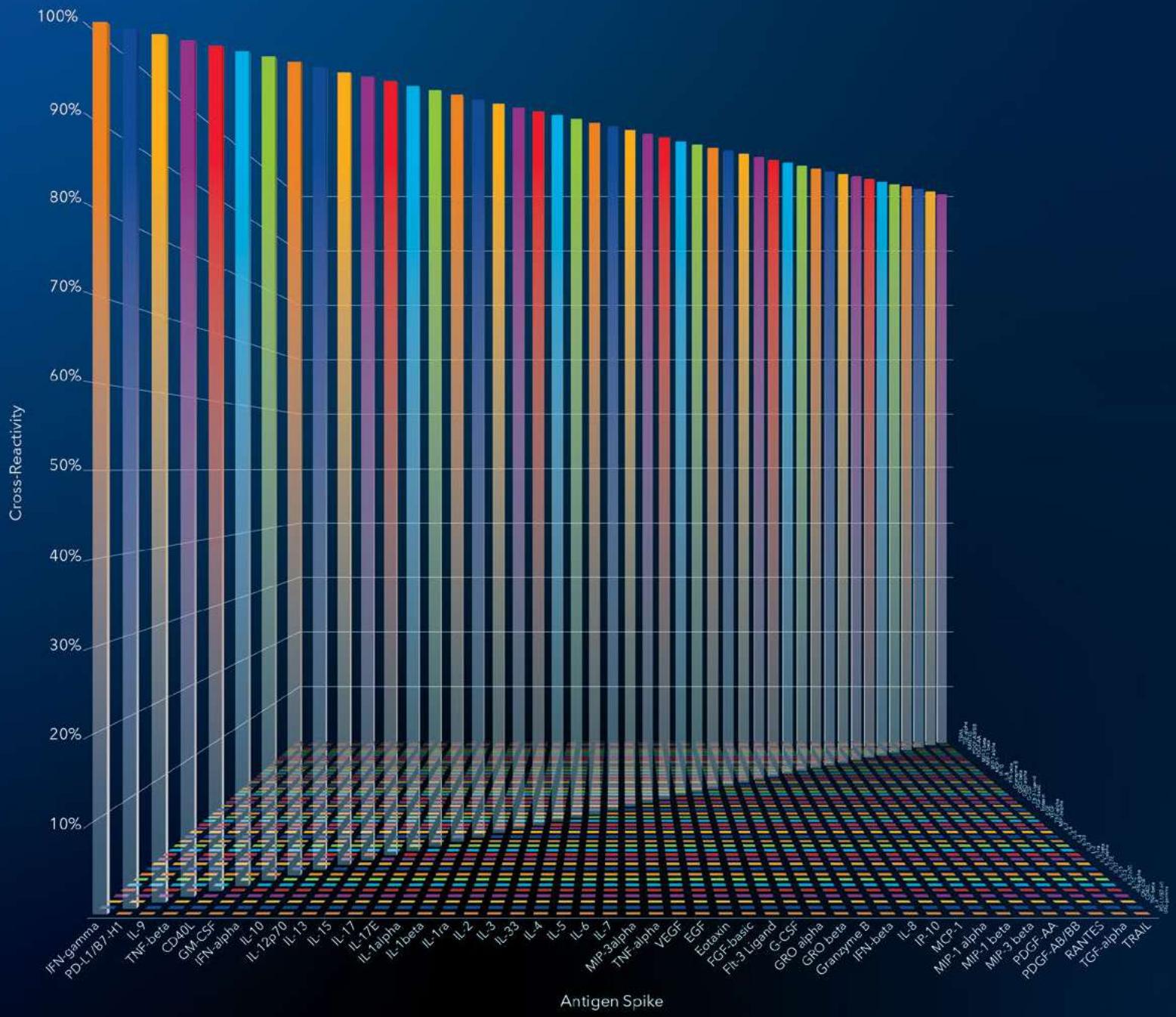


Figure 6. Luminex® Assay Cross Reactivity. Human XL Cytokine Performance Luminex Panel (Catalog #FCSTM18B) cross-reactivity was evaluated by testing recombinant stocks of each antigen. Antigens were spiked in at 3-times the value of the high standard and tested for cross-reactivity within the panel. Less than 0.5% cross-reactivity was measured across all 46 analytes, confirming high panel specificity.

Antigen **Cross-Reactivity** Testing

Cross-reactivity occurs when a molecule other than the analyte of interest is bound by both antibodies leading to a false positive result.

R&D Systems regularly evaluates molecules related to the analyte for cross-reactivity and includes the findings in product data sheets where appropriate.

We also carefully optimize our in-house antibodies to avoid cross-reactivity with substances in the sample. We exhaustively test our antibody pairs against related epitopes to ensure analyte specificity.

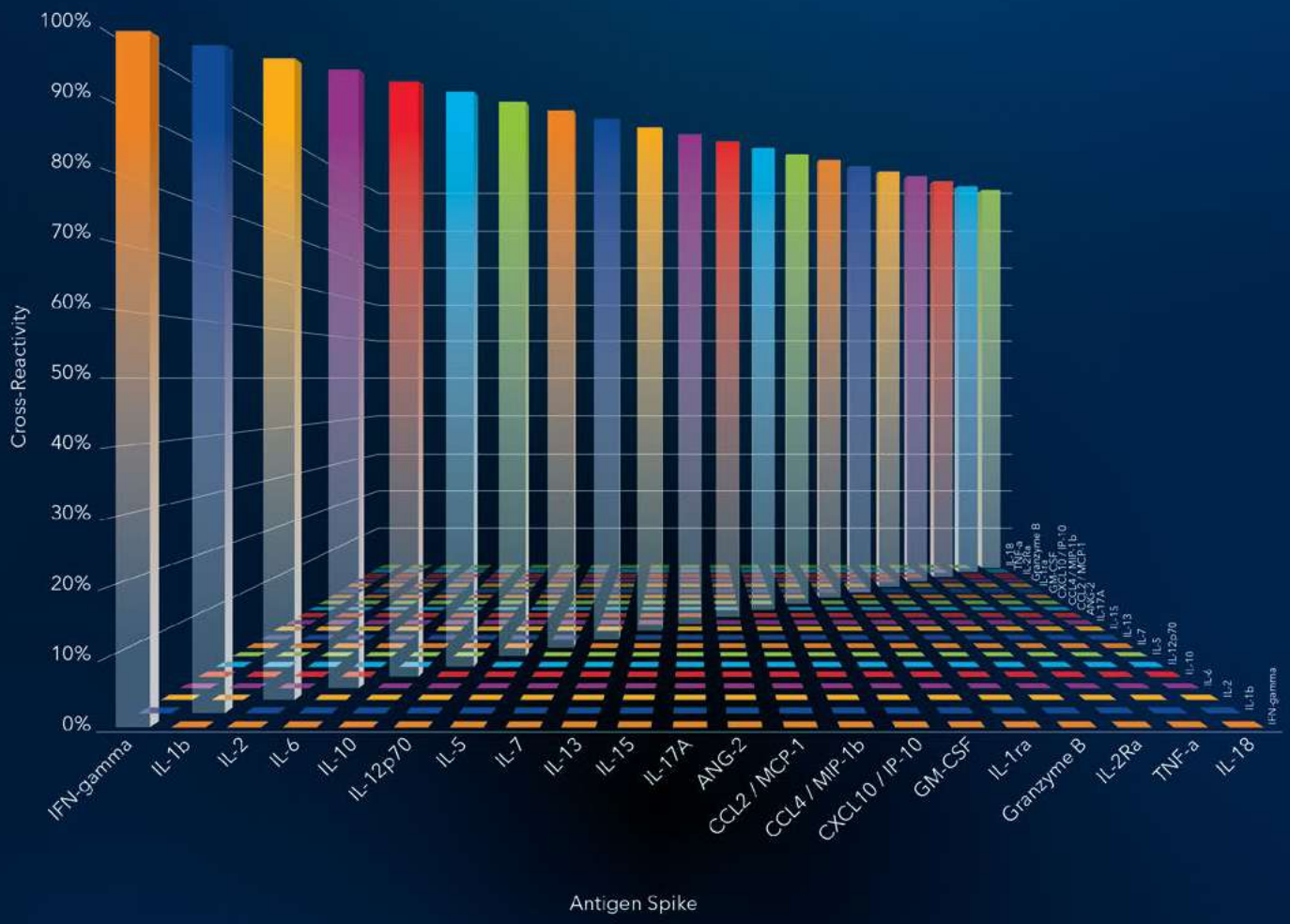


Figure 7. Simple Plex Assay Cross Reactivity. In Simple Plex multiplex assays, each microfluidic channel contains four Glass Nano Reactors (GNRs) allowing for simultaneous quantification of up to two analytes per channel for a total of eight analytes per cartridge. Analyte pairings were validated to ensure detection antibody cross-reactivity or interference does not occur within the channel. Detection antibodies for analyte pairs were mixed and tested with each individual capture antibody. Endogenous samples were measured for detectable RFU values. No false positive or negative results were seen for the pairings shown in the figure. Incompatible pairings are not included in the same channel.

Consistency

Consistency, or reproducibility, of a method measures precision.

R&D Systems assays ensure lot-to-lot consistency, enabling long-term research studies. This, combined with our stringent manufacturing and quality control measures, ensures reliable and reproducible results between kit lots and prevents sample value drift.

Extensive quality control testing is done on each kit component and each complete kit to make sure standard curves, background, and sample values remain consistent.

Factory calibration is used to prevent variability over time due to differences in protein immunoreactivity. Each new standard lot is calibrated against a gold-standard factory calibrator which is manufactured during assay development.

Where appropriate for serum and plasma samples, natural matrix controls are used to adjust standard curves to minimize issues with commutability and lot-to-lot variation.

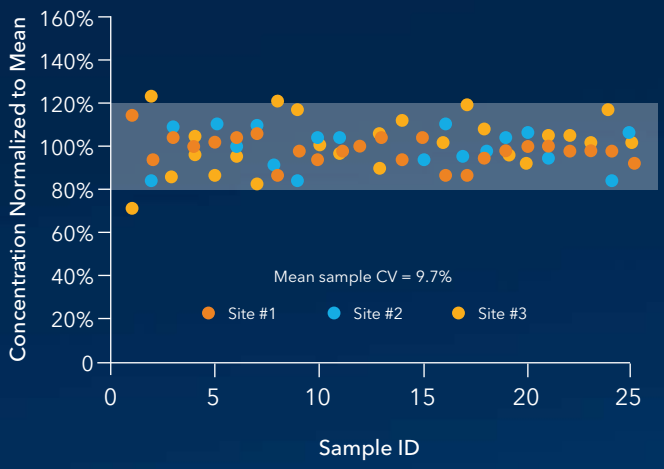


Figure 8. Simple Plex Assays are Consistent Across Users and Sites. Plasma and serum samples, obtained from healthy donors, were prepared according to the Simple Plex NF-L Assay (Catalog #SPCKB-PS-002448) instructions and measured at three different sites. The mean sample coefficient of variation (CV) was less than 10%. This data indicates that Simple Plex assays are consistent across geographies and users.

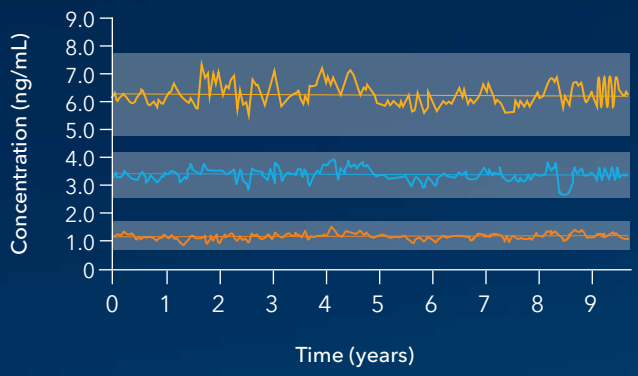


Figure 9. Long-term ELISA Consistency. High (yellow line), medium (blue line), and low (orange line) controls are assayed with every manufactured lot of the Human Fractalkine ELISA Kit (Catalog #DCX310). Controls fall within acceptable ranges (gray bars) and remain consistent from lot to lot over 10 years.

The Right **Immunoassay Platform** When You Need It

INSTRUMENT	FORMAT	KIT	NUMBER OF ANALYTES	BENEFIT	SAMPLE VOLUME	ASSAY TIME
ELISA Plate Reader	96-well plate	 Quantikine ELISA	1	Most published Gold standard Low CVs	10-200µL	3-5 hrs
		 Quantikine QuicKit		Single wash step Results in 90 minutes	50µL	1.5 hrs
		 Quantikine High Sensitivity ELISA		Highest sensitivity	10-200µL	4.5 hrs
Ella	Cartridge	 Simple Plex Assays	up to 8	Easiest workflow Hands free Low user variability	2.5-25µL	1.5 hrs
Luminex	Bead-based	 Luminex High Performance Assays	up to 46	Completely optimized panels	25µL	4 hrs
		 Luminex Discovery Assays	up to 50	Most flexible panels	25µL	4 hrs

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