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Biologics Done Right

A Fully-Validated 10-analyte Multiplex for Quantitation of Human Cytokines

Background

Eurofins Pharma Bioanalytical Services is a biologics-focused, global leader in bioanalytical solutions providing over 20 years of industry-leading scientific expertise in comprehensive PK/TK, ADA, Nab, Biomarker assays and sample analysis for the world's largest pharmaceutical and biopharmaceutical companies.

Sample Analysis at the

RIGHT TIME & RIGHT PLACE

to satisfy data delivery requirements

Background

Cytokines are a broad category of small proteins that are generally associated with the immune system and can have a major impact on autoimmune diseases, cancer and other inflammatory conditions in humans. The role of cytokines in the drug development process is ever expanding as they may be the target of a new drug within a specific condition or conversely, can influence the efficacy of the drug in a positive or negative manner.

Pharmaceutical researchers are routinely using these cytokine measurements as biomarkers of drug efficacy. The ability to measure multiple cytokines within a single sample, single well, is quite advantageous to the researcher. The drug development process requires years of preclinical and clinical studies for drug approval. As part of this process, well characterized analytical assays are needed for those biomarkers and in many cases, are expected to be validated to ensure data reproducibility and integrity to support drug submission.

V-PLEX validated assays deliver reproducibility and reliability to support the most demanding long-term studies. Developed under design control and according to the FDA's analytical validation guidelines, V-PLEX represents the highest quality assay available from MesoScale Discovery (MSD). Comprehensive testing of all raw materials and kit components combined with rigorous manufacturing and QC specifications ensure reproducible results time after time.

ANALYTES

IFN-γ

IL-1β

IL-2

IL-4

IL-6

IL-8

IL-10

IL-12p70

IL-13 TNF-α

Results

ACCURACY & PRECISION

Validation samples were created by spiking cytokines into Assay Diluent at Lower Limit of Quantitation (LLOQ), Low, Mid, High and Upper Limit of Quantitation (ULOQ) levels, the concentrations of which varied per analyte. These samples were assayed in seven runs, with three reportable results for each level, to assess Accuracy and Precision.

Analyte	LLOQ (pg/mL)	Inter- Assay Precision (%CV)	Inter- Assay Accuracy (%RE)	ULOQ (pg/mL)	Inter- Assay Precision (%CV)	Inter- Assay Accuracy (%RE)
IFN-γ	10.6	5.8	-8.4	1058.3	5.1	-0.3
IL-1β	4.1	4.5	-7.6	406.7	3.4	0.6
IL-2	10.2	4.4	-4.2	1016.7	5.8	-0.5
IL-4	2.0	5.3	-5.4	200.0	5.3	-0.3
IL-6	5.2	5.0	-7.4	517.5	5.3	0.0
IL-8	4.2	8.1	-6.3	420.0	5.3	1.0
IL-10	2.6	6.1	-8.4	255.8	7.1	-0.1
IL-12p70	3.2	7.0	-6.7	324.2	7.2	-2.0
IL-13	4.3	6.0	-22.8	426.7	3.6	1.3
TNF-α	2.6	8.6	-23.7	260.8	5.4	-5.7

TABLE I. Accuracy and Precision at Lower (LLOQ) and Upper (ULOQ) Limits of Quantitation

SELECTIVITY

Selectivity was determined by spiking analytes at or near the MQC level into 10 different samples of human serum, and assaying those along with unspiked samples in two separate runs. The recovery was calculated after accounting for endogenous analyte.

Analyte	Lowest recovery (%RE)	Highest recovery (%RE)	Proportion of lots < +/- 25% RE (number)
IFN-γ	-26.4	-5.4	8 of 10
IL-1β	-18.1	-5.4	10 of 10
IL-2	-33.1	15.9	9 of 10
IL-4	-38.4	-5.7	7 of 10
IL-6	-26.3	-11.4	9 of 10
IL-8	-10.6	5.7	9 of 9
IL-10	-24.8	1.1	10 of 10
IL-12p70	-9.0	16.7	10 of 10
IL-13	-48.5	15.9	9 of 10
TNF-α	-13.2	14.8	10 of 10

TABLE 2. Selectivity in human serum.

ENDOGENOUS PRECISION

To test endogenous precision, 5 serum samples from healthy individuals and 5 samples from diseased individuals were tested over 6 assay runs.

Analyte	Inter-Assay Precision (%CV)*
IFN-γ	7.4
IL-6	7.1
IL-8	5.9
TNF-α	13.9

TABLE 3. Endogenous Precision. * Average %CV of 10 samples over 6 assays. Majority of samples for IL-1β, IL-2, IL-4, IL-10, IL-12p70 and IL-13 were <LLOQ.

Background

DILUTIONAL LINEARITY

Linearity of dilution was evaluated by testing each analyte at two concentrations above the standard curve range, to evaluate hook effect, and at three dilutions within the curve range, to test linearity.

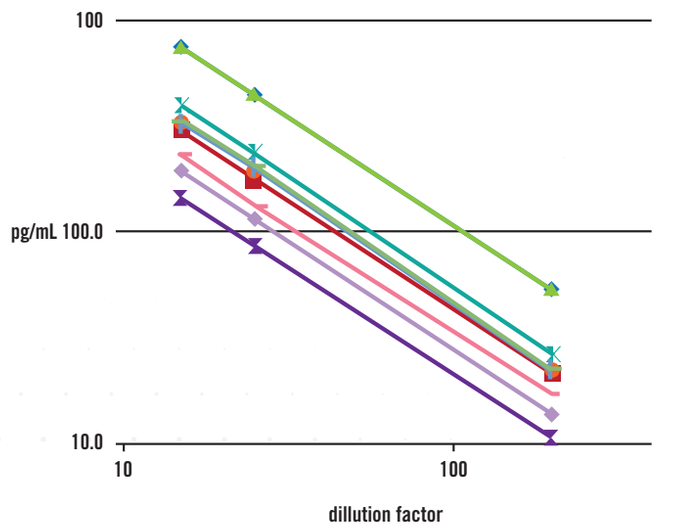

FIGURE 1. Dilutional Linearity

Validation Conclusions

The Proinflammatory I V-Plex panel from Mesoscale Discovery was successfully validated to a regulatory-compliant level. Inter-assay accuracy and precision were evaluated, with relative error (RE) within +/- 25% and CVs less than 25%, for all 10 analytes. Selectivity was acceptable, with at least 8 of 10 samples yielding >75% spike recovery for all analytes except IL-4, for which 7 of 10 samples were acceptable. Dilutional linearity was also demonstrated for all 10 analytes.

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Immunogenicity

- Assay development, method transfer, validation. Cut-point calculation
- Screen, Confirm, Titer. Advanced methodologies to address drug tolerance and soluble target interference
- Advanced cell based laboratory dedicated to GLP NAb assay development and sample analysis
- Experience and capacity for large volume sample analysis

Biosimilars

- Pre-developed assays for Trastuzumab, Bevacizumab, Cetuximab, Adalimumab, and more
- PK, Immunogenicity evaluation
- FcRN, Fc RI, II, III and C1q binding
- Cell based assays
- Receptor binding, proliferation, ADCC

Biomarkers

- GLP/GCP/CLIA assay development, method transfer, validation
- Exploratory sample analysis using kits from any vendor across a wide variety of platforms
- Luminex, ELISA, RIA, MSD, GyroLab, Flow Cytometry, Singulex
- Full flow cytometry capabilities to support GxP studies
- Immunophenotyping, pharmacodynamics
- Cytokine release assays

Pharmacokinetics

- Large molecule specialists, capacity for large volume sample analysis
- Clinical and Pre-clinical PK studies
- Exploratory / GxP
- Latest platforms including GyroLab and Singulex

Contact us today to discover how the Eurofins team can make the difference in your projects.

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About Eurofins Bioanalytical Services

Over 20 years of industry-leading global **Scientific Expertise** supporting the widest breadth of Biologics' clinical trials with PK/TK, ADA, Nab and Biomarker assays and sample analyses.

Versatile Performance and Project Management Excellence to adapt to a client's specific needs. Clinical or preclinical, regulated or non-regulated, assay development, qualification or validation; we custom design our support to match the client's program.

State-of-the art laboratory facilities in St. Louis, USA providing **Global Reach and Capacity** to address clients' needs while simultaneously offering regionally-based solutions.

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