Development and Validation of Multiplexed Non-Human Primate Cytokine V-PLEX® Panels

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Sensitivity and Assay Protocol

The lower limit of detection (LOD) is a calculated concentration corresponding to the signal-to-noise standard deviation above background noise. The LOD is determined as the value at which signal-to-noise is 3, or the minimum signal above assay background noise. Sensitivity is calculated as the lower limit of detection (LOD) divided by the recovery of the analyte within the range of 80% to 120% of the known value (75% to 125% for IL-8). The data are from >30 runs collected over at least 2 months by 4 operators. The NHP cytokine multiplex panel was developed as three panels designed for optimal performance based on individual assay characteristics, native analyte levels and suitable dilution, diluent, and assay compatibility. The Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits are based on MSD® ECL technology and follow the same simple three-step protocol.

Methods

V-PLEX technology (V-PLEX (design), and SPOT THE DIFFERENCE) is a highly sensitive, high-throughput technology that utilizes a chemiluminescent signal amplification in combination with microplate technology to achieve high performance in cytokine arrays. Electrochemiluminescence (ECL) enables signal amplification, which leads to ultrasensitive detection and superior sensitivity. Instead of washing, non-washed assays enable rapid readout times and improve sensitivity.

Electrochemiluminescence Technology

- Based on the ECL principle using luminol as the chemiluminophore
- Luminol is excited by microwave radiation
- Luminol reacts with hydrogen peroxide in the presence of an oxidation catalyst to produce chemiluminescence
- Signal increases up to 10-fold

V-PLEX technology and format offers competitive advantages in terms of sensitivity and precision. ECL technology offers advantages over other signal detection technologies such as fluorometric and chemiluminescent: ECL elicits strong signal and long signal duration, allows for lower detection limits, is free of photobleaching, has lower background signal, and can be used for simultaneous detection of multiple analytes.

Development and Validation

To evaluate the ability of our antibody pairs to recognize native analytes in cynomolgus and rhesus matrices, we screened normal cynomolgus and rhesus matrices (obtained from a commercial vendor) for native analyte levels using the Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP), and Chemokine Panel 1 (NHP) panels. Normal of samples were detected using IL-6, IL-8, or IL-12 samples. Results are shown in Table 1. All controls tested were within the target range of 80% to 120% of assigned concentration (dashed lines). Only labels bound near the electrode surface are excited, enabling non-washed assays.

Spike Recovery and Dilution Linearity

Spike recovery testing was conducted in NHP serum, heparin plasma, EDTA plasma, whole plasma, urine and cell culture media. Representative data are shown in Table 2. Recovery values were obtained through freeze–thaw testing and accelerated stability studies for calibrators, antibodies, and controls. The validation program includes a real-time stability study with scheduled performance evaluations of complete kits for at least 30 months from date of manufacture.

Calibration Curves

Calibration curves were generated for each assay. The curves were generated by taking the average of at least 25 samples conducted by 3 operators on a minimum of 3 days.

Reproducibility

To evaluate the consistency of the panels, four levels of standards spanning the quantifiable range were prepared and measured in separate runs for each panel. Data were obtained from Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits. The average Fold Dilution Recovery values were within 10% of the target range of 100%+/-5%.

Conclusions

This multiplex panel has been analytically validated for use in the quantification of NHP cytokines and chemokines simultaneously in whole plasma, sera, and culture supernatants. The panel is highly sensitive, able to detect low levels of cytokines and chemokines, and has excellent reproducibility and linearity. The panel is highly reproducible and widely applicable to multiple non-human primate (NHP) models. We report the validation of multiple V-PLEX cytokine assays for applications in rhesus and cynomologus models. We also evaluated the ability of our antibody pairs to detect NHP analytes in the supernatants of activated cynomologus or rhesus peripheral blood lymphocytes (PBLs). PBLs were treated with nothing, ConA, LPS, PHA or PWM for 6, 24, or 48 hours. The stimulated samples were tested on Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits. Results of the panel were compared to baseline samples of unstimulated PBLs. Results were evaluated with both all samples measured within 10% of expected concentration levels. Results from all panels were analyzed using standard statistical methods.

Stimulated Sample Testing

To evaluate the ability of our antibody pairs to recognize native analytes in cynomolgus and rhesus matrices, we screened normal cynomolgus and rhesus matrices (obtained from a commercial vendor) for native analyte levels using the Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP), and Chemokine Panel 1 (NHP) panels. Normal of samples were detected using IL-6, IL-8, or IL-12 samples. Results are shown in Table 1. All controls tested were within the target range of 80% to 120% of assigned concentration (dashed lines). Only labels bound near the electrode surface are excited, enabling non-washed assays.

Conclusion

Multiplex panels have been developed and validated, tailored for use in the quantification of NHP cytokines and chemokines simultaneously in whole plasma, sera, and culture supernatants. The panel is highly sensitive, able to detect low levels of cytokines and chemokines, and has excellent reproducibility and linearity. The panel is highly reproducible and widely applicable to multiple non-human primate (NHP) models. We report the validation of multiple V-PLEX cytokine assays for applications in rhesus and cynomologus models. We also evaluated the ability of our antibody pairs to detect NHP analytes in the supernatants of activated cynomologus or rhesus peripheral blood lymphocytes (PBLs). PBLs were treated with nothing, ConA, LPS, PHA or PWM for 6, 24, or 48 hours. The stimulated samples were tested on Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits. Results of the panel were compared to baseline samples of unstimulated PBLs. Results were evaluated with both all samples measured within 10% of expected concentration levels. Results from all panels were analyzed using standard statistical methods.

Figure captions:

- Sensitivity and Assay Protocol: The lower limit of detection (LOD) is a calculated concentration corresponding to the signal-to-noise standard deviation above background noise. The LOD is determined as the value at which signal-to-noise is 3, or the minimum signal above assay background noise. Sensitivity is calculated as the lower limit of detection (LOD) divided by the recovery of the analyte within the range of 80% to 120% of the known value (75% to 125% for IL-8). The data are from >30 runs collected over at least 2 months by 4 operators. The NHP cytokine multiplex panel was developed as three panels designed for optimal performance based on individual assay characteristics, native analyte levels and suitable dilution, diluent, and assay compatibility. The Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits are based on MSD® ECL technology and follow the same simple three-step protocol.

- Methods: The V-PLEX multiplex panels were developed as three panels designed for optimal performance based on individual assay characteristics, native analyte levels and suitable dilution, diluent, and assay compatibility. The Proinflammatory Panel 1 (NHP), Cytokine Panel 1 (NHP) and Chemokine Panel 1 (NHP) kits are based on MSD® ECL technology and follow the same simple three-step protocol.