Optimal Detectability with Luminex Based Multiplex Cytokine Assay on Wall Less Plates: A Case Validation Study with Experimental Autoimmune Encephalomyelitis Mouse Plasma Samples

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PURPOSE:

Luminex® multiplex cytokine assay is an effective platform for Biomarker discovery but display a limiting sample volume requirement of 25µl/well which can be constraining with precious samples such as small biopsies, gingival crevicular fluid, tears, mouse cerebrospinal fluid... Luminex multiplex assay shows high performance with cell supernatant samples but in certain instances such as with complex body fluid, sample analysis may lead to sub-optimal analyte detection and necessitate an optimal choice of standard cytokine concentration curve range for maximising all analytes detection.

METHODS:

DA-Bead is a wall less 96 well Droparray plate which handles small reaction volume of 5-20µl in a drop format(Fig 1A). The unique engineered design of Droparray's plate has drops contained within a hydrophilic area which is surrounded by hydrophobic material. Each circular hydrophilic area receives conventional Luminex reagents such as xMAP® magnetic beads/antibody/sample/standards in a minimal drop of 5µl volume with 80% miniaturization as compared to traditional volume. DA-Bead is used sequentially on vortex/orbital shakers and washed in a fully automated station(Fig 1B). Here, we present out a parallel evaluation of classical and DA-Bead method with a Milliplex® based workflow with plasma samples obtained from a mouse model of Experimental Autoimmune Encephalomyelitis(EAE) subjected to treatment with dexamethasone(DXM).

Figure 1: (A) Curiox DA-Bead 96 well wall less plate. (B) Curiox LT-MX washer

A

DA-Bead 96 well plate holding 5µl drops/well respectively.

B

Table 1: MilliPlex standard cytokine key performance parameters comparison between conventional vs DA-Bead Method

	Limit of Quantitation					
	Low(pg/ml)*		High(pg/ml)*		Intra-Assay %CV*	
Analytes	DA-Bead	Conventional	DA-Bead	Conventional	DA-Bead	Conventional
IL-25	585.9	585.9	600000	600000	11.7	13.3
GM-CSF	34.2	34.2	8750	8750	10.1	18.6
IFN-γ	7.8	7.8	8000	8000	9.0	11.2
Mip-3a	48.8	48.8	3125	3125	16.5	15.3
IL-1β	14.7	14.7	15000	15000	7.9	15.1
IL-2	5.9	5.9	6000	6000	3.5	20.3
IL-4	1.5	1.5	1500	1500	9.3	18.9
IL-5	19.5	19.5	5000	5000	18.3	15.9
IL-6	31.3	7.8	8000	8000	13.4	19.1
IL-21	19.5	19.5	20000	5000	11.3	16.3
IL-22	2.4	2.4	2500	2500	9.5	17.8
IL-28β	127.0	127.0	8125	8125	6.6	12.6
IL-10	78.1	19.5	20000	20000	8.4	12.3
IL-23	341.8	341.8	350000	350000	9.3	17.2
IL-12p70	19.5	19.5	20000	5000	7.4	17.4
IL-27	878.9	878.9	900000	900000	12.6	13.5
IL-13	39.1	156.3	40000	40000	8.9	12.2
IL-15	34.2	34.2	35000	35000	7.5	17.1
IL-17A	39.1	39.1	40000	40000	9.1	19.1
IL-17F	9.8	9.8	10000	10000	10.3	12.8
IL-33	78.1	78.1	80000	20000	7.6	16.0
IL-31	48.8	48.8	50000	50000	7.2	9.6
TNFβ	488.3	488.3	500000	500000	12.3	14.4
TNFα	3.4	3.4	3500	875	8.2	22.9
CD40L	48.8	48.8	50000	50000	8.2	17.5

*Lower and higher limit of quantitation was defined as a reliable quantitative range where values could be estimated with 70-130% recovery and a precision below 25 %CV

RESULTS:

Upon using Millliplex based kits with standard cytokine we obtained with DA-Bead method a precision below 20% CV, accuracy within 70-130% recovery and reliable LLOQ sensitivity in similar levels with conventional methods. Both methods displayed good correlation with cytokine standards(R^2=0.99)(Fig 2A) and mouse plasma samples(R^2=0.93)(Fig 2B). Plasma sample analysis from an EAE model on DA-Bead method highlighted decreased instances of non detection/out of ranges occurrences as compared to conventional method(Fig 2C). Improvement in detectability with DA-Bead method generated an increase in statistical significance of modulated plasma samples cytokine levels and identified a new responsive role of dexamethasone in modulating IL-17F/IL-17-A in EAE mouse model(Fig 3).

Figure 2:(A) Cytokine Standards Correlation, (B) Plasma Samples Correlation (C) Detectability statistics comparison with both Luminex methods.

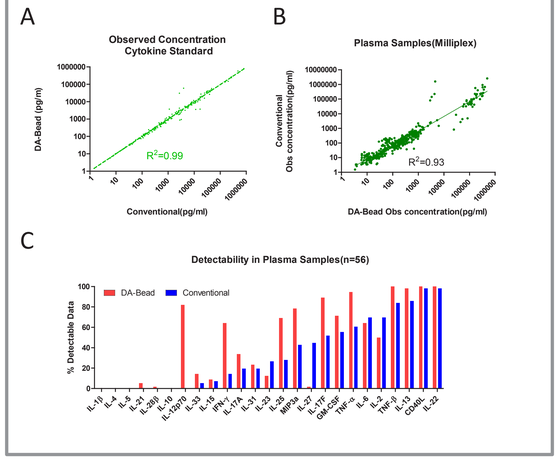
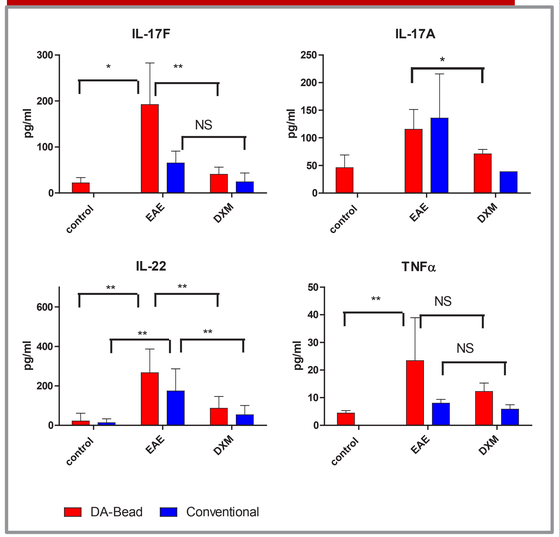


Figure 3: Functional luminex cytokine mouse plasma sample analysis comparison of both methodologies with EAE mouse study and dexamethasone as a drug candidate.



CONCLUSION:

- DA-Bead method is equivalent in performance to traditional method but use 5x Less sample and reagent volumes..
- Detectability with DA-Method and mouse plasma samples in Luminex assay is significantly enhanced in this EAE trial.
- DA-Bead method highlights a new role for dexamethasone in suppressing IL-17F and IL-17A cytokine level in EAE context.

With improvement in performance over conventional plates, DA-Bead is the next generation Luminex assay plate for maximising results with smallest sample volume.