Evaluation of Commercially Available Luminex Multiplex Kits

Program # 268

Joanne Lannigan, M.S. and Michael Solga, M.S.
Flow Cytometry Core, University of Virginia, Charlottesville, VA

IINTRODUCTION

There are many choices of Luminex based multiplex kits commercially available. As part of a U19 Multi-Investigator Multi-Center Program we sought to evaluate the performance of several commercially available Luminex multiplex kits to determine which kits would provide the most reliable data for this program.

MATERIALS AND METHODS

Intra-assay CV, inter-assay CV, percent recovery, the linearity and consistency of the standard curves, linearity of a serial dilution, lower limit of detection, and any cross-reactivity between analytes were measured. Initial studies were conducted using kits from Millipore, Affymetrix, Invitrogen, BioLegend, and R&D Systems . The cytokines GM-CSF, IL-1α, IL-1β, IL-2, IL-4 and TNF-α were selected as a representative sample for testing. Cytokine standards with known concentration were obtained from the National Institute for Biological Standards and Control. Kits were tested as follows: each of the cytokines individually, all of the cytokines together, a 4-step 1:10 serial dilution of the combined cytokine sample, mouse serum alone, and mouse serum spiked with the combined cytokine sample. 2.5ng/mL was selected as the primary testing concentration as it is relatively near the middle of the standard curve for most of the kits and analytes. The individual cytokine tests were used to identify cross-reactive bead sets and to measure the average intra-assay CV and % recovery. The combined cytokine test was used to measure the average intra-assay CV and % recovery and to calculate the inter-assay CV. The serial dilution was used to identify the lower limit of detection. The 1:10 and 1:100 dilutions of the combined cytokine sample were used to measure the linearity of each kit within the range of 25pg/mL to 2500pg/mL. The 1:10 dilution was used in the average intra-assay CV and % recovery of the serum sample and cytokine spiked serum samples.

RESULTS

Linearity of Standard Curve

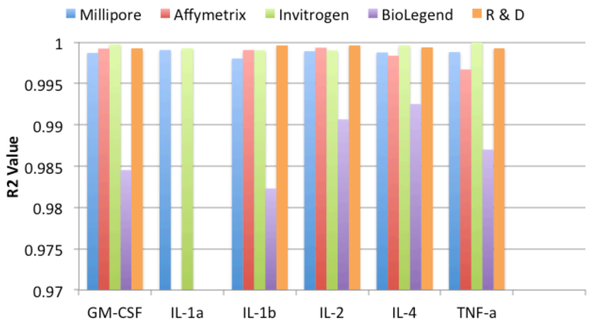


Figure 1. All of the kits provided reasonable linearity with 6-8 point standard curves, however BioLegend's were lower than all the others.

Linearity of 1:10 Serial Dilutions

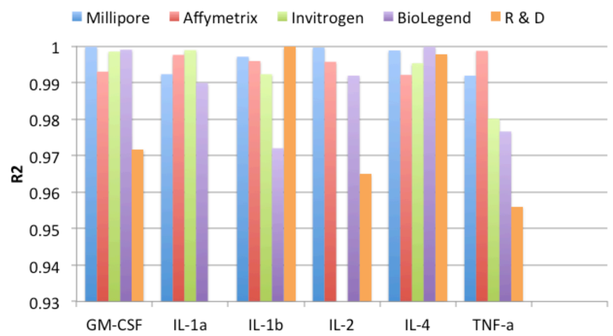
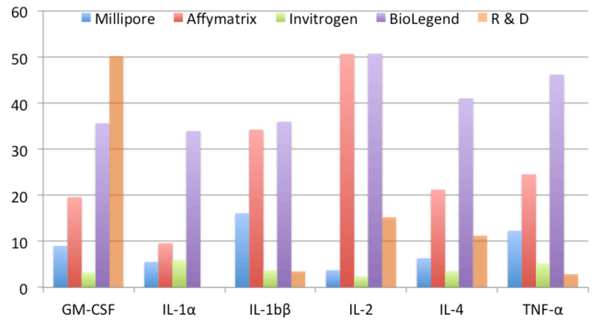


Figure 2. Using 10 fold serial dilutions reasonable linearity was obtained on all kits, however, BioLegend, Invitrogen & R&D had decreased linearity on several analytes

Minimal Detectable Concentration

Manufacturer	Minimal Detectable Concentration (pg/ml						Comments
	GM-CSF	IL-1a	IL-1b	IL-2	IL-4	TNF-a	
Millipore	11.2	10.2	2	0.8	0.2	1	
Affymetric	-	-	-	-	-	-	Not Provided
Invitrogen	<20	<30	<20	<40	<10	<10	
BioLegend	0.8	NP	1	0.6	0.4	3.6	
R & D	-	-	-	-	-	-	Not Provided

Intra-Assay Variability (%CV)



Inter-Assay Variability (%CV)

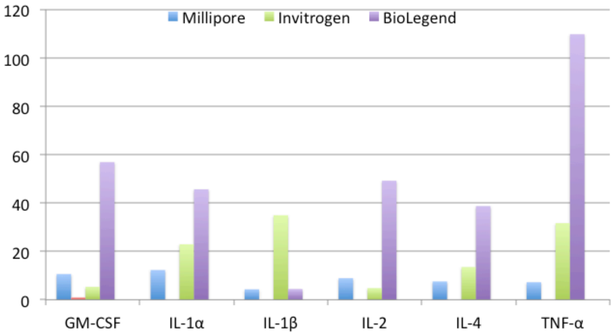


Figure 3 and 4. BioLegend and Affymetrix kits gave the largest intra assay variability; Millipore had the best reproducibility for both intra and inter-assay variability.

% Recovery

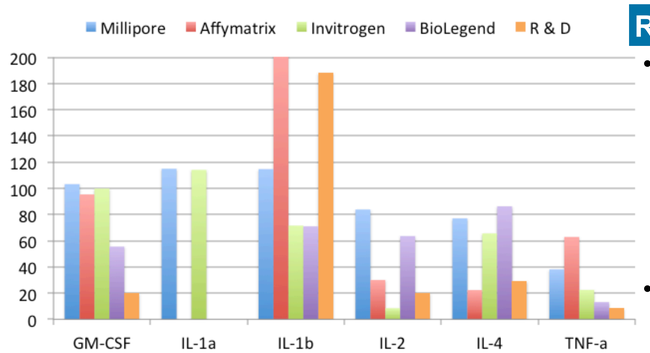


Figure 5. Millipore kits provided the best percent recovery with the exception of TNF-α, however, all kits had low % recovery for TNF-α.

Specificity (Cross-reactivity pg/ml))

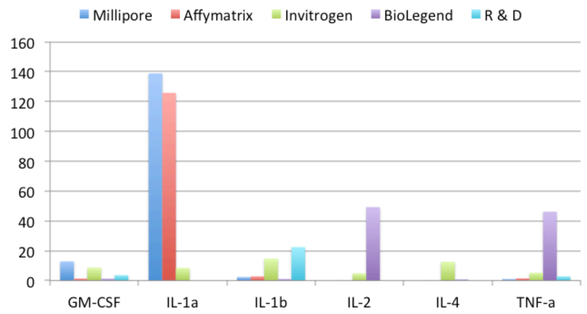


Figure 6. Specificity was high for all kits with the exception of IL-1α; both Millipore and Affymetrix kits had reasonably high cross-reactivity with this analyte. This cross-reactivity was eliminated in the Millipore kit (but not the Affymetrix kit) with the use of a serum matrix as a diluent.

CONCLUSION

Of all the variables tested the Millipore kits gave the best overall performance. In addition, the ability to order kits pre-mixed or as individual bead analytes, the option for an overnight incubation, the provision of excess reagent, the inclusion of high and low internal controls and outstanding technical customer support, supported the decision for these kits to be the kit of choice.

REFERENCES

- ***WHO cytokine standardization: facilitating the development of cytokines in research, diagnosis and therapeutic agents.*** Mire-Suis, AR, Gaines Das, R and Padilla, A; J. Immunol. Methods 216, 1998 103-116.
- ***Immunoassays for detecting cytokines: What are they really measuring? .*** Mire-Suis, AR, Gaines Das, R and Thorpe, R; J. Immunol. Methods 186, 1995a, 161-164,