Comparison of serum cytokine measurement techniques between ELISA vs Multiplex

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ABSTRACT

Background: Enzyme-linked immunosorbent assay (ELISA) has been classically described as the gold standard for serum cytokine analysis. However, multiplex array technologies are increasing in popularity because of their ability to analyze large numbers of analytes using low sample volume compared to ELISA. However, few studies have directly compared serum cytokine results from ELISA and multiplex analytical techniques. **Purpose:** To determine differences in serum cytokine concentrations between ELISA and Multiplex techniques. **Methods:** Blood samples were collected from the antecubital vein of professional American football players during a competitive season. After clotting, blood was centrifuged and three 300 µL aliquots of serum were frozen at -80˚ C. IL-1β, IL-6, and TNF-α were measured using ELISA (EMD Millipore, Sigma Aldrich) and Multiplex (EMD Millipore, MagPix) kits. For standardization, one technician carried out the respective protocol under the same laboratory conditions on the same samples. Data are shown as mean±SD. Coefficients of variation (CV%), paired samples t-test with 95% CI, and Pearson's product-moment correlations were used to compare cytokine analysis techniques. **Results:** Serum cytokine concentrations were not comparable between ELISA and Multiplex analytical techniques. IL-1β (n=18) displayed significant variability between techniques with a CV% of 119.9 with ELISA reading 36.8±32.4 pg/mL, intra-assay CV%=7.6 and Multiplex showing 1.1±1.2 pg/mL, intra-assay CV%=6.8. IL-6 (n=33) demonstrated substantial variability between techniques with a CV% of 126.9 with ELISA reading 340.2±460.5 pg/mL, intra-assay CV%=15.3 and Multiplex showing 11.8±20.2 pg/mL, intra-assay CV%=4.8. For TNF-α (n=36) all ELISA samples were below the detection limit of 0.31 with the Multiplex detecting 8.8±3.2 pg/mL, intra-assay CV%=7.9. Moreover, paired samples t-tests showed considerable mean differences between analysis techniques for IL-1β (-35.8 pg/mL, (95% CI, -51.6 to -19.9) t(17)=-4.8, p<0.001) and IL-6 (-328.4 pg/mL (95% CI,-490.0 to -166.7), t(32)=-4.1, p<0.001. Pearson's correlation between ELISA and Multiplex was not significant for IL-1β [r(18)=0.44, p=0.068] or IL-6 [r(33)=0.25, p=0.157]. **Conclusion:** The results suggest that differences in blocking agents between both techniques may be the source of erraticism. According to the outcomes of this study, comparisons between analytical techniques should be avoided.

BACKGROUND/PURPOSE

Enzyme-linked immunosorbent assay (ELISA) has been classically described as the gold standard for serum cytokine analysis. However, multiplex array technologies are increasing in popularity because of their ability to analyze large numbers of analytes using low sample volume compared to ELISA. However, few studies have directly compared serum cytokine results from ELISA and multiplex analytical techniques.

The purpose of this study was to determine differences in serum cytokine concentrations between ELISA and Multiplex techniques.

METHODS

Blood samples were collected from the antecubital vein of professional American football players during a competitive season. After clotting, blood was centrifuged and three 300 µL aliquots of serum were frozen at -80˚ C. IL-1β, IL-6, and TNF-α were measured on a multi-plate reader (Biotek, Cytation 3) using ELISA (EMD Millipore, Sigma Aldrich) assay and measured on a Multiplex reader (EMD Millipore, MagPix) using Milliplex map assay kits. For standardization, one technician carried out the respective protocols on each instrument under the same laboratory conditions for the same samples. Data are shown as mean±SD. Coefficients of variation (CV%), paired samples t-test with 95% CI, and Pearson's product-moment correlations were used to compare cytokine analysis techniques.

Figure 1. Luminex MagPix

Image from: emdmillipore.com

Figure 2. Biotek Cytation 3

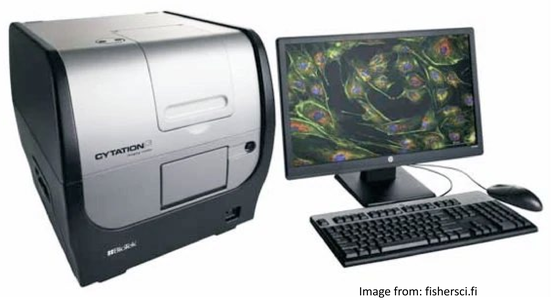


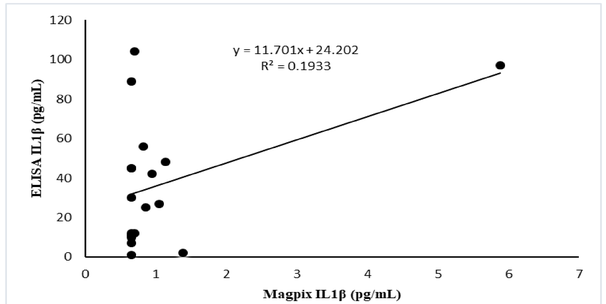
Image from: fishersci.fi

RESULTS

Table 1. Comparison between ELISA and Magpix IL-1β

Descriptive Statistics	ELISA IL-1β (pg/mL)	Magpix IL-1β (pg/mL)
Mean ± SD (pg/mL) *(n=18)*	1.1 ± 1.2	36.8 ± 32.4
Intra-assay CV (%)	6.8	7.6
Between Methods CV (%)	119.9	

Figure 3. ELISA IL-1β (pg/mL) vs. Magpix IL-1β (pg/mL)

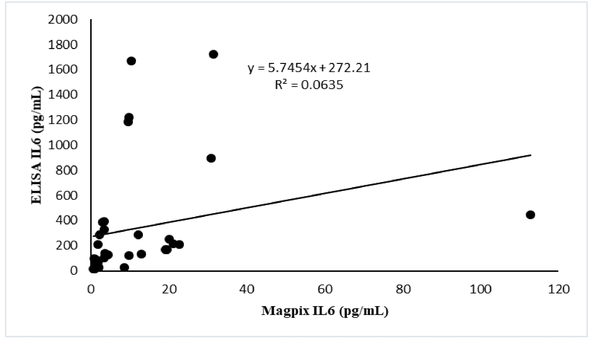


Pearson's correlation shows that there were no significant correlation between ELISA and Multiplex for IL-1β [r(18)=0.44, p=0.068].

Table 2. Comparison between ELISA and Magpix IL-6

Descriptive Statistics	ELISA IL-6 (pg/mL)	Magpix IL-6 (pg/mL)
Mean±SD (pg/mL) *(n=33)*	340.2±460.5	11.8±20.2
Intra-assay CV (%)	15.3	4.8
Between Methods CV (%)	126.9	

Figure 4. ELISA IL-6 (pg/mL) vs Magpix IL-6 (pg/mL)



Pearson's correlation shows that there were no significant correlation between ELISA and Multiplex for IL-6 [r(33)=0.25, p=0.157].

RESULTS

Table 3. Paired Samples t-test between ELISA and Magpix IL-1β and IL-6

Paired Samples t-test
IL-1β (-35.8 pg/mL, (95% CI, -51.6 to -19.9) t(17)=-4.8, p<0.001)
IL-6 (-328.4 pg/mL (95% CI,-490.0 to -166.7), t(32)=-4.1, p<0.001)

Paired samples t-tests showed considerable mean differences between techniques for IL1β p<0.001) and IL6 p<0.001.

Serum cytokine concentrations were not comparable between ELISA and Multiplex analytical techniques. IL-1β (n=18) displayed significant variability between techniques with a CV% of 119.9 with ELISA reading 36.8±32.4 pg/mL, intra-assay CV%=7.6 and Multiplex showing 1.1±1.2 pg/mL, intra-assay CV%=6.8. IL-6 (n=33) demonstrated substantial variability between techniques with a CV% of 126.9 with ELISA reading 340.2±460.5 pg/mL, intra-assay CV%=15.3 and Multiplex showing 11.8±20.2 pg/mL, intra-assay CV%=4.8. For TNF-α (n=36) all ELISA samples were below the detection limit of 0.31 with the Multiplex detecting 8.8±3.2 pg/mL, intra-assay CV%=7.9. Moreover, paired samples t-tests showed considerable mean differences between analysis techniques for IL-1β (-35.8 pg/mL, (95% CI, -51.6 to -19.9) t(17)=-4.8, p<0.001) and IL-6 (-328.4 pg/mL (95% CI,-490.0 to -166.7), t(32)=-4.1, p<0.001. Pearson's correlation between ELISA and Multiplex was not significant for IL-1β [r(18)=0.44, p=0.068] or IL-6 [r(33)=0.25, p=0.157].

CONCLUSIONS

The results suggest that differences in blocking agents between both techniques may be the source of erraticism. According to the outcomes of this study, comparisons between different analytical techniques for biomarker analysis should be avoided.

Additional research is needed to evaluate the causes of these discrepancies, as well as, further comparisons of additional biomarkers and manufacturers.

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