IL-6 VALIDATIONS

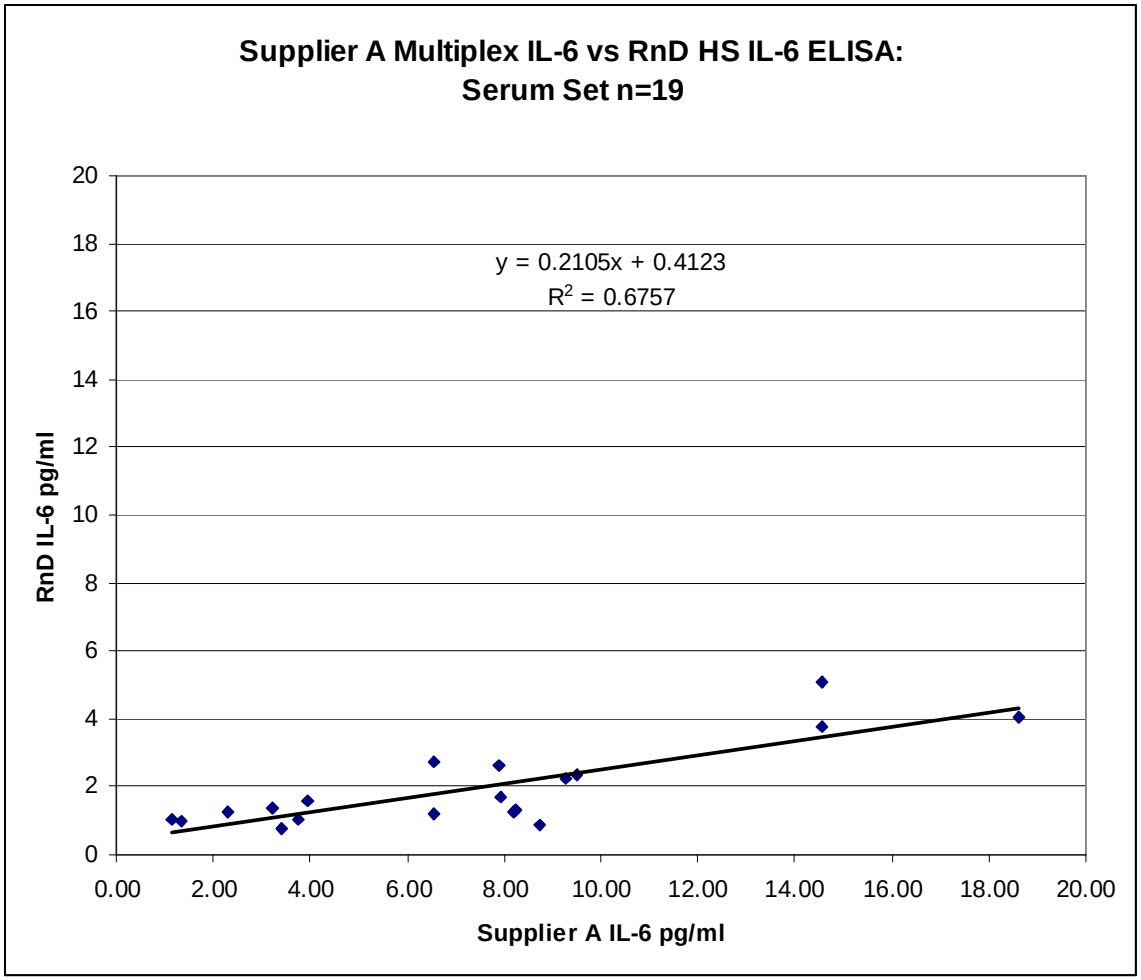
Several suppliers' reagents correlate well with R&D Systems HS ELISA, the gold-standard assay normally used in our laboratory.

Assays are not always "harmonized" with respect to standardization. Slope(m) values reflect degree of harmonization.

Suppliers

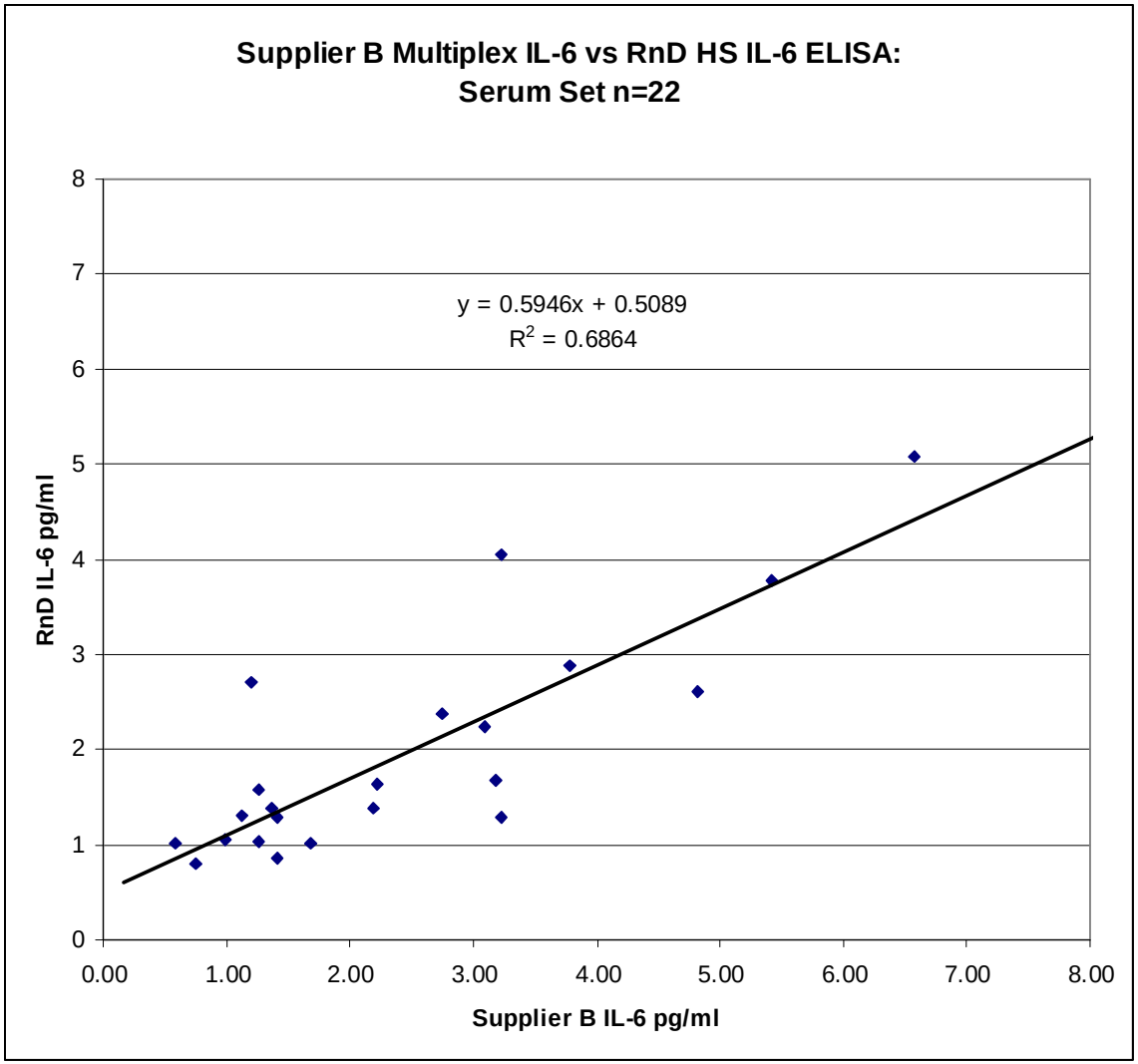
- A: Bio-Rad
- B: Linco Research
- C: Upstate
- D: Biosource
- E: RnD Systems

SUPPLIER A



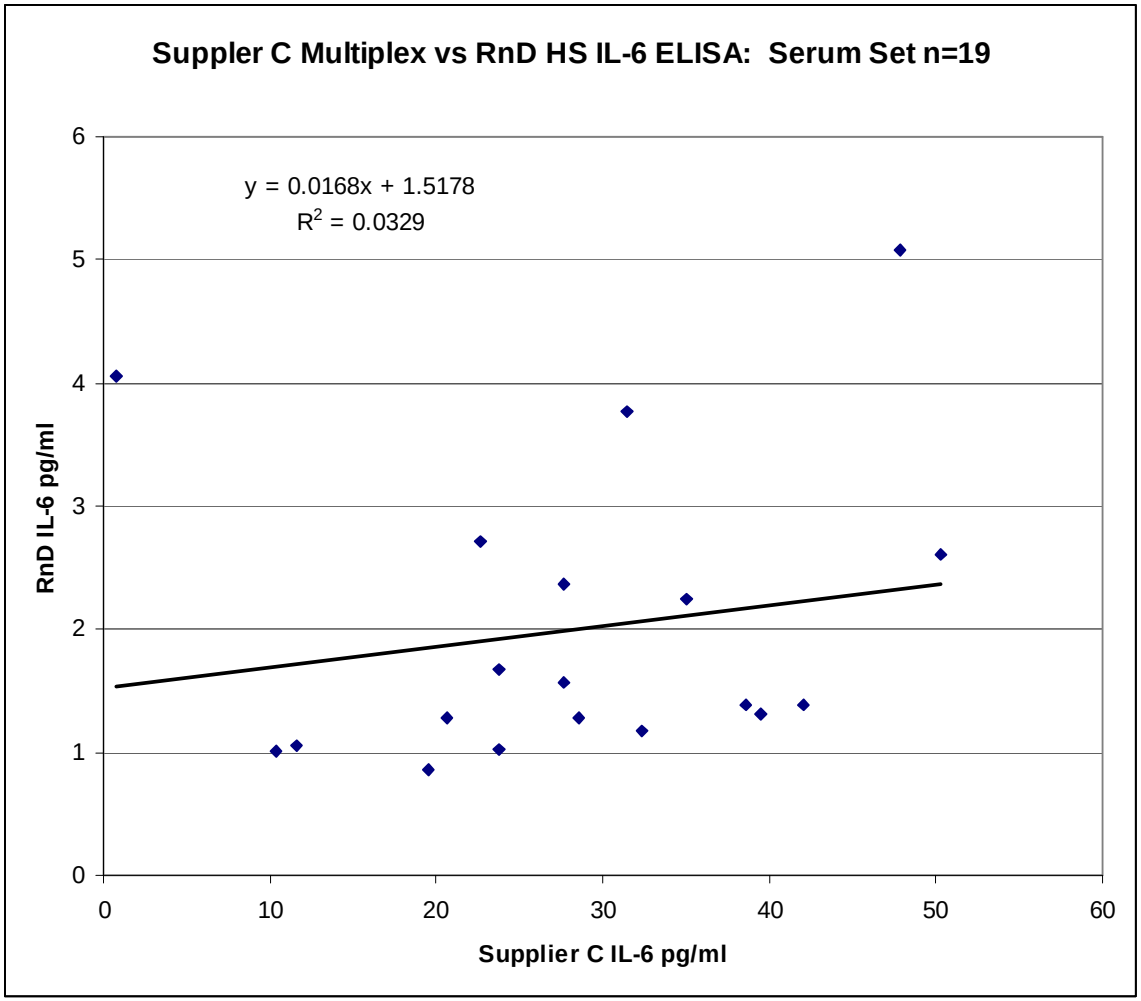
Mean replicate CV=26.6%
R^2=0.68
Slope(m)=0.21

Supplier B



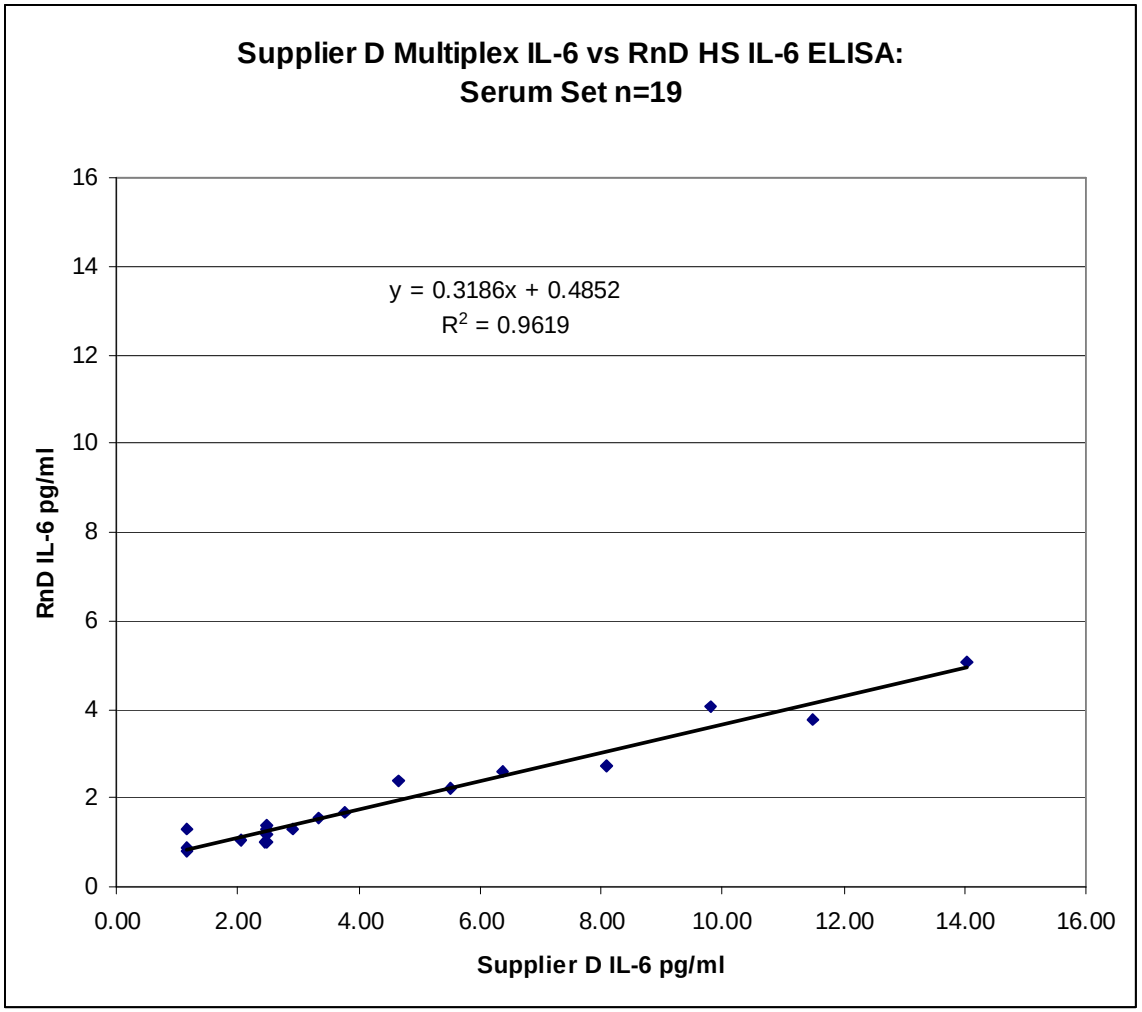
Mean replicate CV=8.9%
$R^2 = 0.69$
Slope(m)=0.59

Supplier C



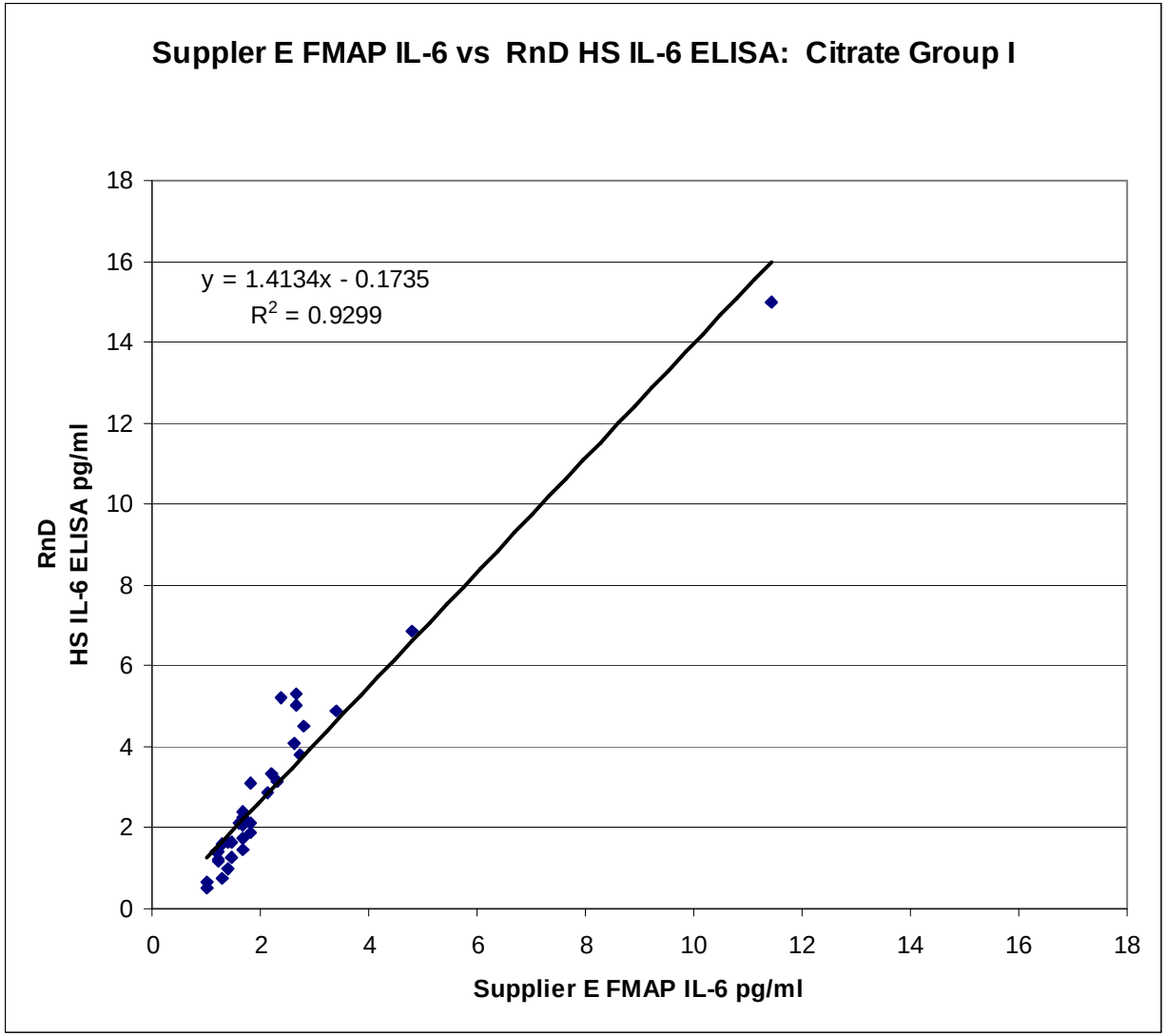
Mean replicate CV=106.9%
R2 = 0.033
Slope(m)=0.017

Supplier D



Mean replicate CV=21.4%
R^2 =0.96
Slope(m)=0.32

Supplier E

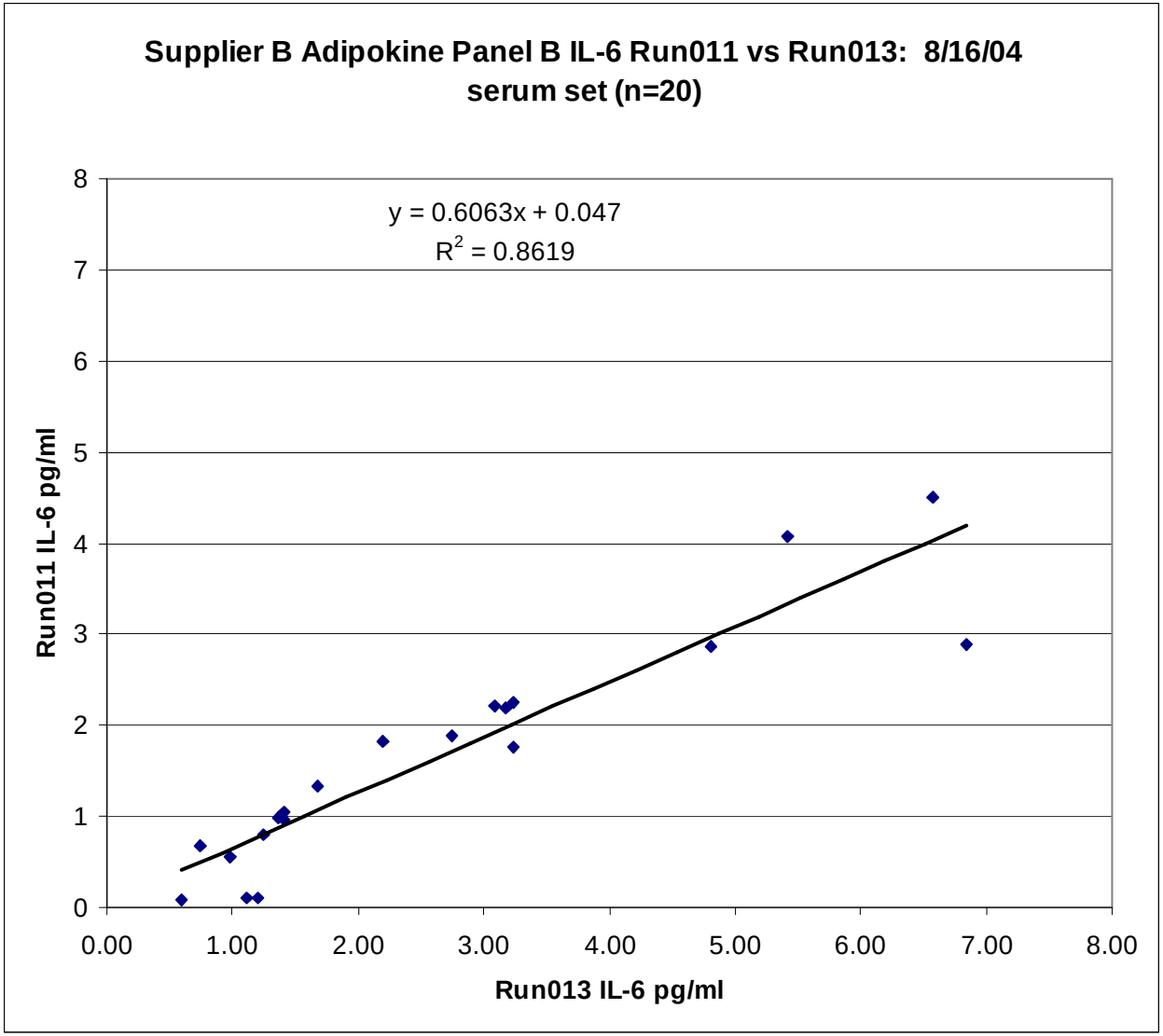


Mean replicate
CV = 24.6%
R^2 = 0.93
Slope(m)= 1.414

Supplier B

- The following 3 slides show run to run reproducibility for Adipokine Panel B IL-6, with assays performed using different kit lots:
- Run011 (9-4-04) vs Run013 (9-29-04)
- Run018 (10-29-04) vs Run013 (9-29-04)
- Run058 (5-24-05) vs Run013 (9-29-04)
- The last slide shows the presence of outlier points caused by heterophilic antibody reaction in Supplier B's IL-6 assay. This problem emerged with kit lots we used between March 2005 and May 2005. If the problem is resolved we will update these validations accordingly.

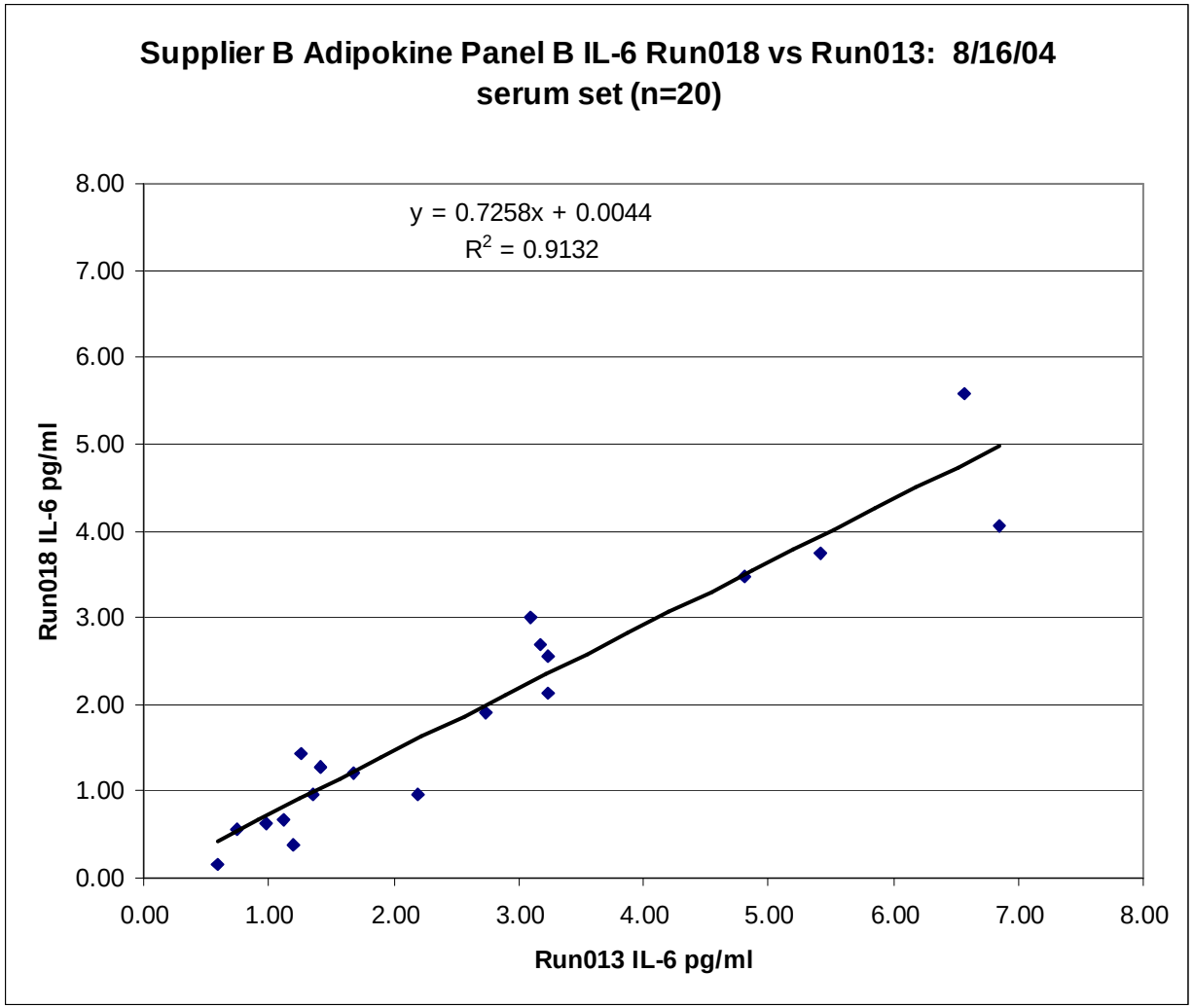
Supplier B



Reproducibility

$R^2 = 0.86$

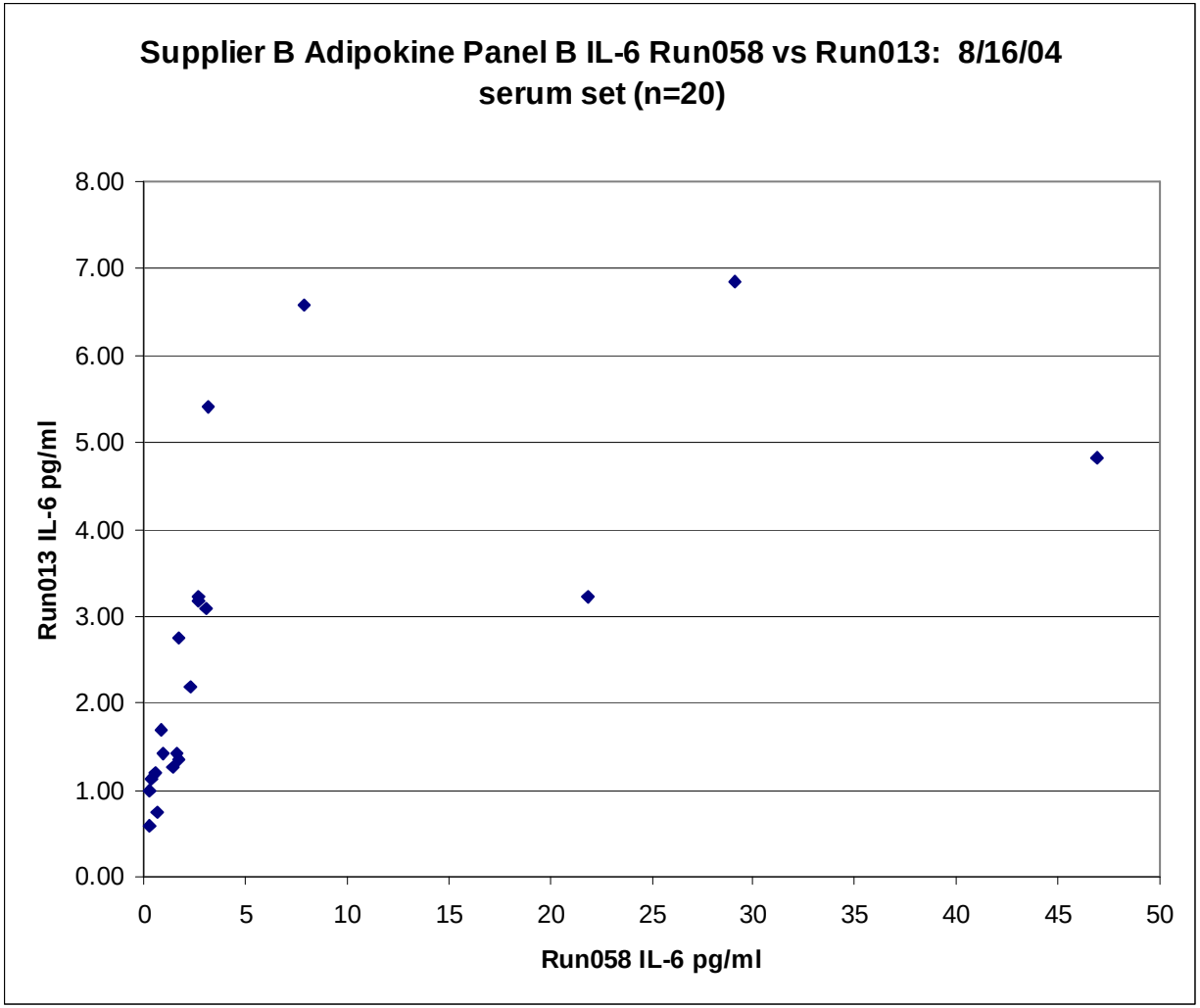
Supplier B



Reproducibility

$R^2 = 0.91$

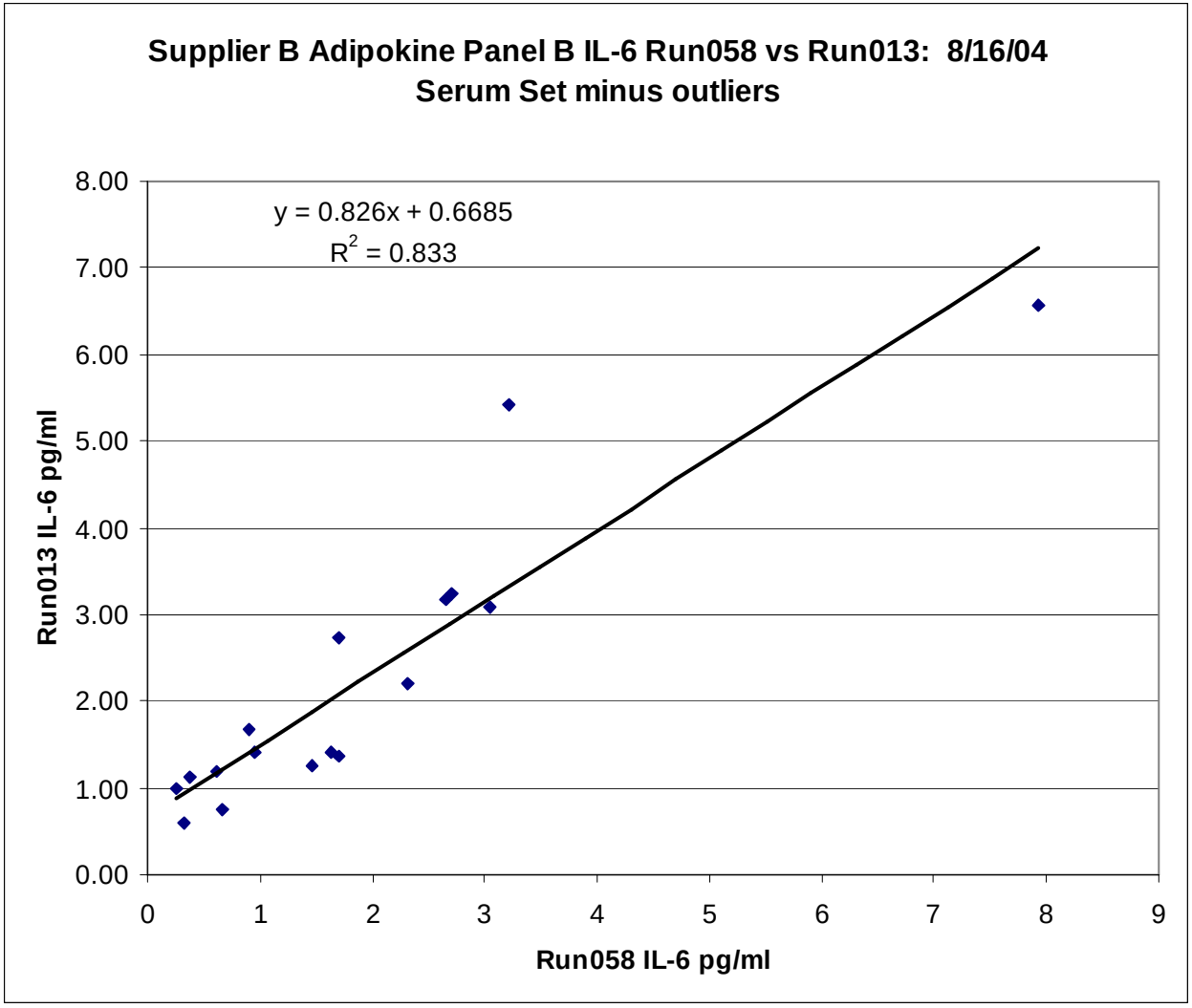
Supplier B



Reproducibility

Shows 3 outlier points caused by heterophilic antibody reaction in Supplier B's May 2005 kit lot.

Supplier B



Reproducibility

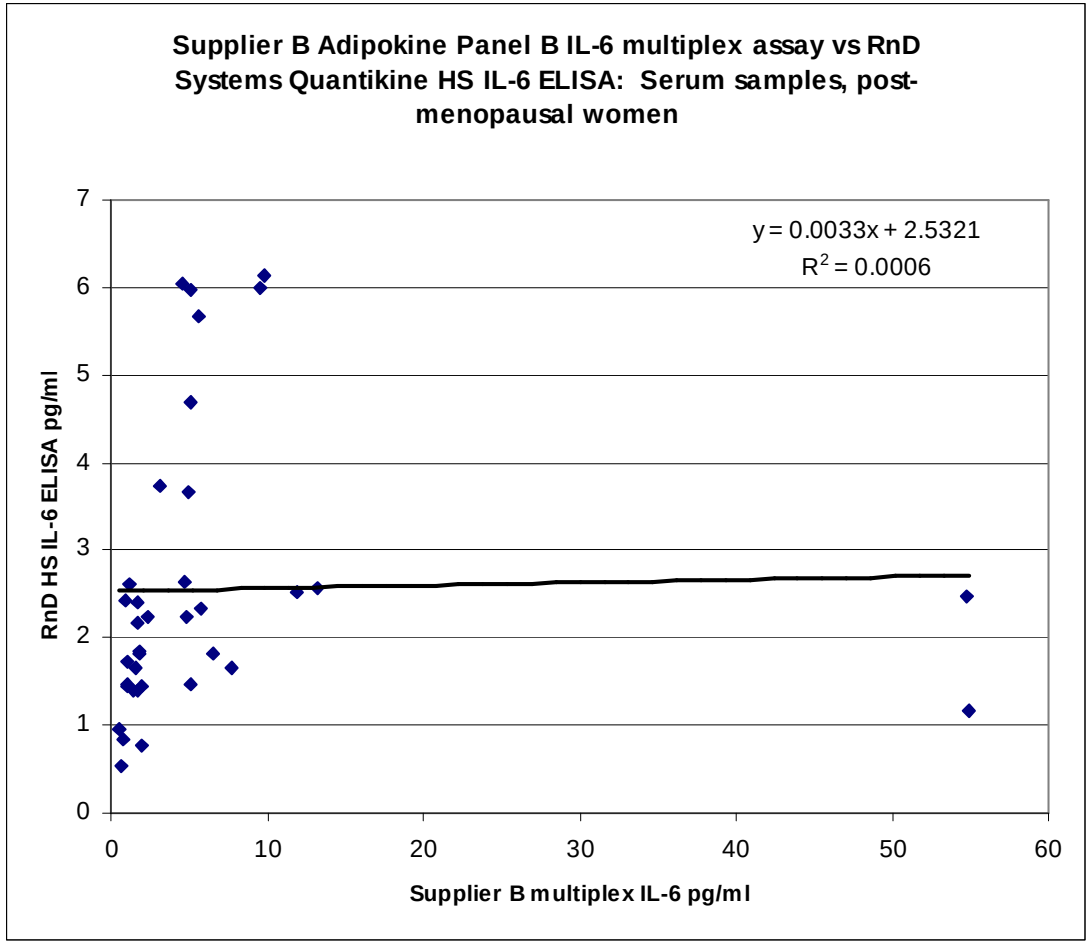
After removal of 3 outlier points, $R^2 = 0.83$, indicating the assay is still working except for these random heterophylic antibody interractions.

Supplier B

- The following slides shows a recent (3/2006) comparison of IL-6 values for supplier B's Adipokine panel B multiplex IL-6 assay with RnD System's Quantikine HS IL-6 ELISA:
- For 36 serum samples from post-menopausal women with two outliers removed, $R^2 = 0.255$, slope = 0.248, indicating the assays shows poor correlation and lack harmonization with respect to standardization.

Supplier B

Supplier B Adipokine Panel B IL-6 vs RnD IL-6 HS ELISA

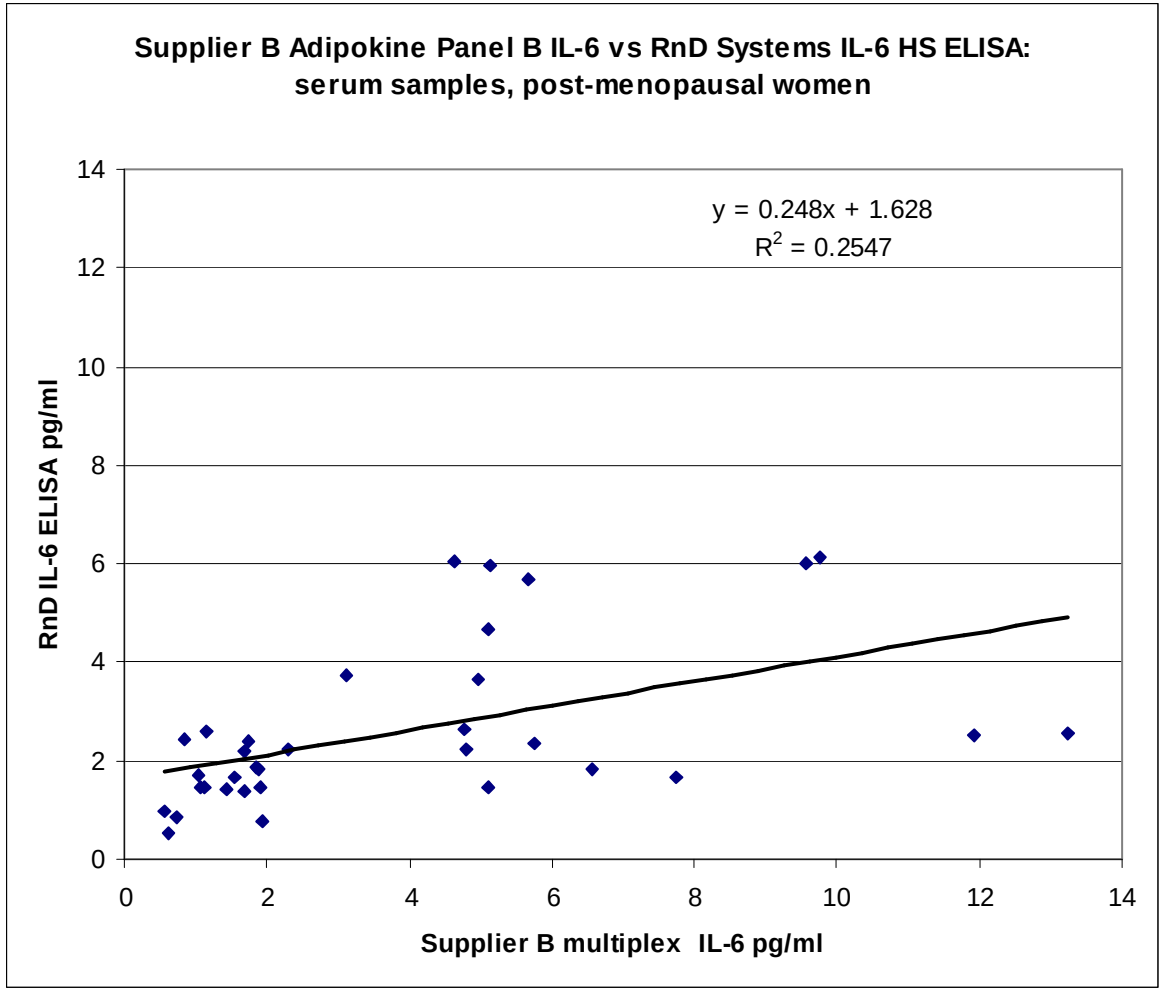


Slope = 0.0033
R^2 = 0.0006

For a recent (3/2006) comparison of Supplier B's Adipokine Panel B IL-6 assay vs RnD System's HS IL-6 ELISA using 36 serum samples from post-menopausal women, there is poor agreement between the assays, slope = 0.248 and R2 = 0.0006. Note that very high values as seen in the two outliers here, should be considered spurious.

Supplier B

Supplier B Adipokine Panel B IL-6 vs RnD IL-6 HS ELISA: Outliers removed



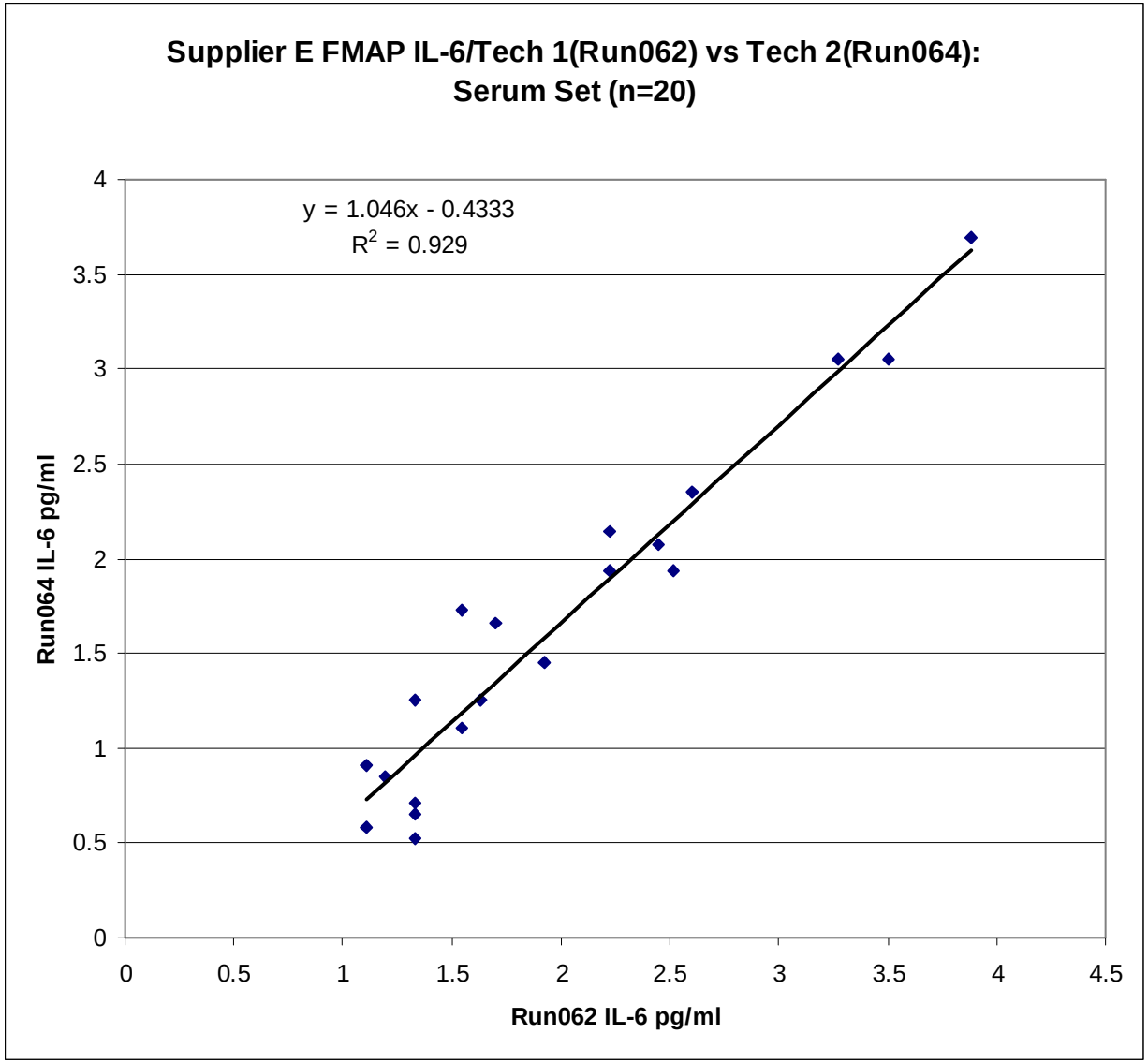
Slope = 0.248
R^2 = 0.255

For a recent (3/2006) comparison of Supplier B's Adipokine Panel B IL-6 assay vs RnD System's HS IL-6 ELISA using 36 serum samples from post-menopausal women, with two outliers removed, R2 = 0.255, showing that there is poor agreement between the assays and that there is a lack of harmonization with respect to standardization (slope = 0.248).

Supplier E

- The following 2 slides show run to run reproducibility for FMAP IL-6, with assays performed by different lab technicians.

Supplier E

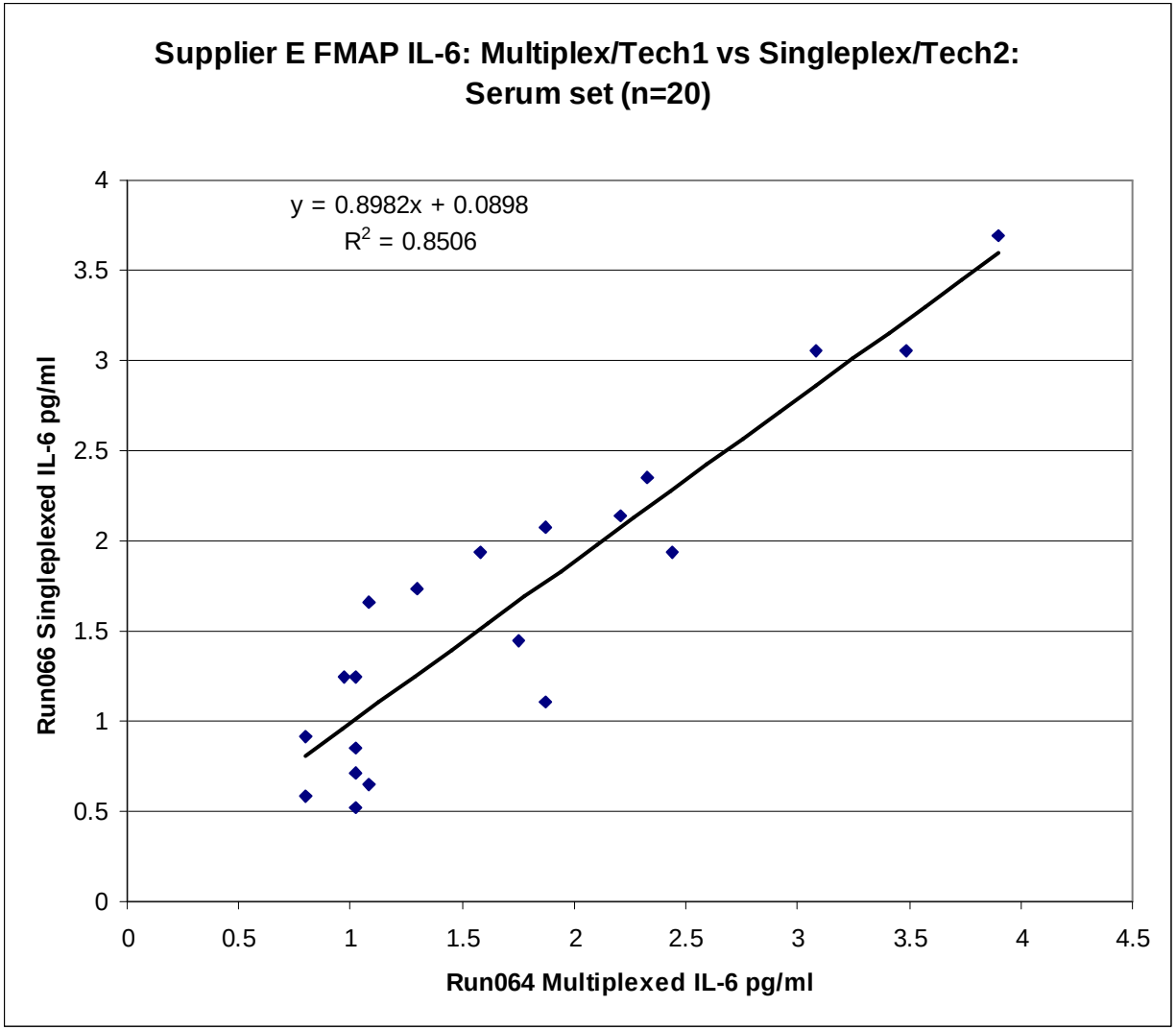


Reproducibility

$R^2 = 0.93$

Note that both runs used multiplexed IL-6 but were performed by different technicians.

Supplier E



Reproducibility

$R^2 = 0.85$

Note that not only do these data reflect multiplexed vs singleplexed IL-6 assays, but assays were performed by different technicians also.