

IL-12p70 Validations

- Validation includes:
- % detectability data for Supplier B's human Cytokine multiplex panels
- Reproducibility data for human Cytokine multiplex panel

Suppliers

- A: Bio-Rad
- B: Linco (done)
- C: Upstate
- D: Biosource
- E: RnD Systems

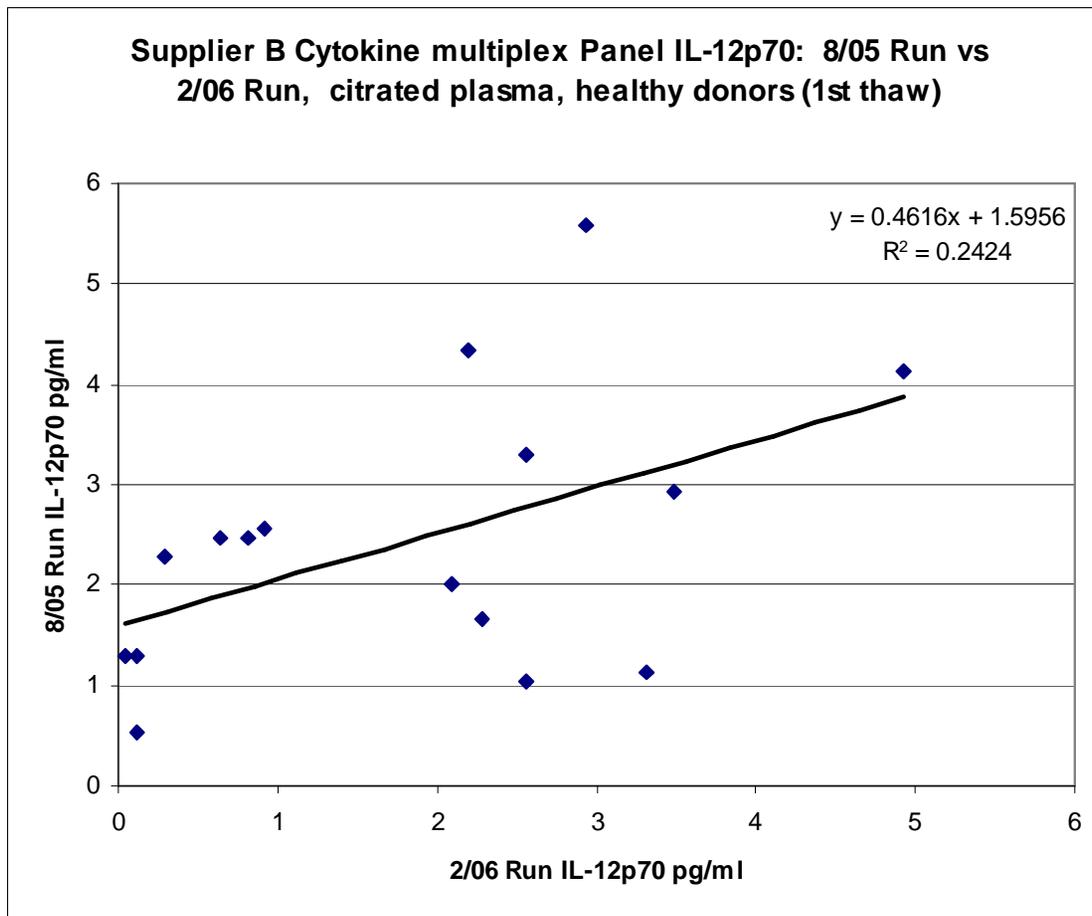
IL-12p70 - % Detectability

- Using supplier B's Cytokine multiplex panel as a 21-plex, % detectability of IL-12p70 in serum and plasma from healthy donors:
- Citrated plasma (n=16): 100%
- Serum (n=20): 80%
- (Note that for each sample type a different set of donors were used.)

Supplier B

Human Cytokine multiplex panel

Reproducibility



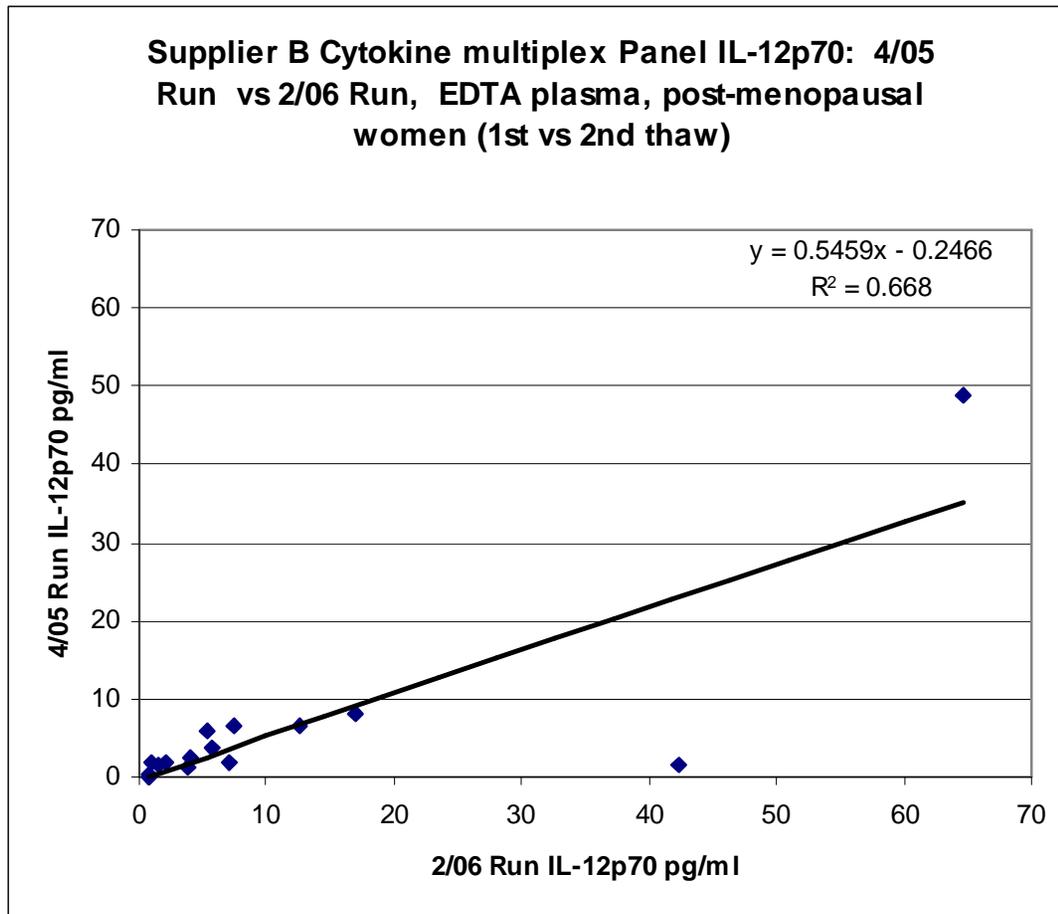
IL-12p70 levels for citrated plasma samples from healthy donors assayed with Supplier B's Cytokine multiplex panel, assayed 8/05 as a 21-plex, showed poor agreement with levels for the same sample set, assayed 2/06 as a 6-plex. Reproducibility is likely affected by levels that approach the lower limit of detectability.

$R^2 = 0.242$

Supplier B

Human Cytokine multiplex panel

Reproducibility



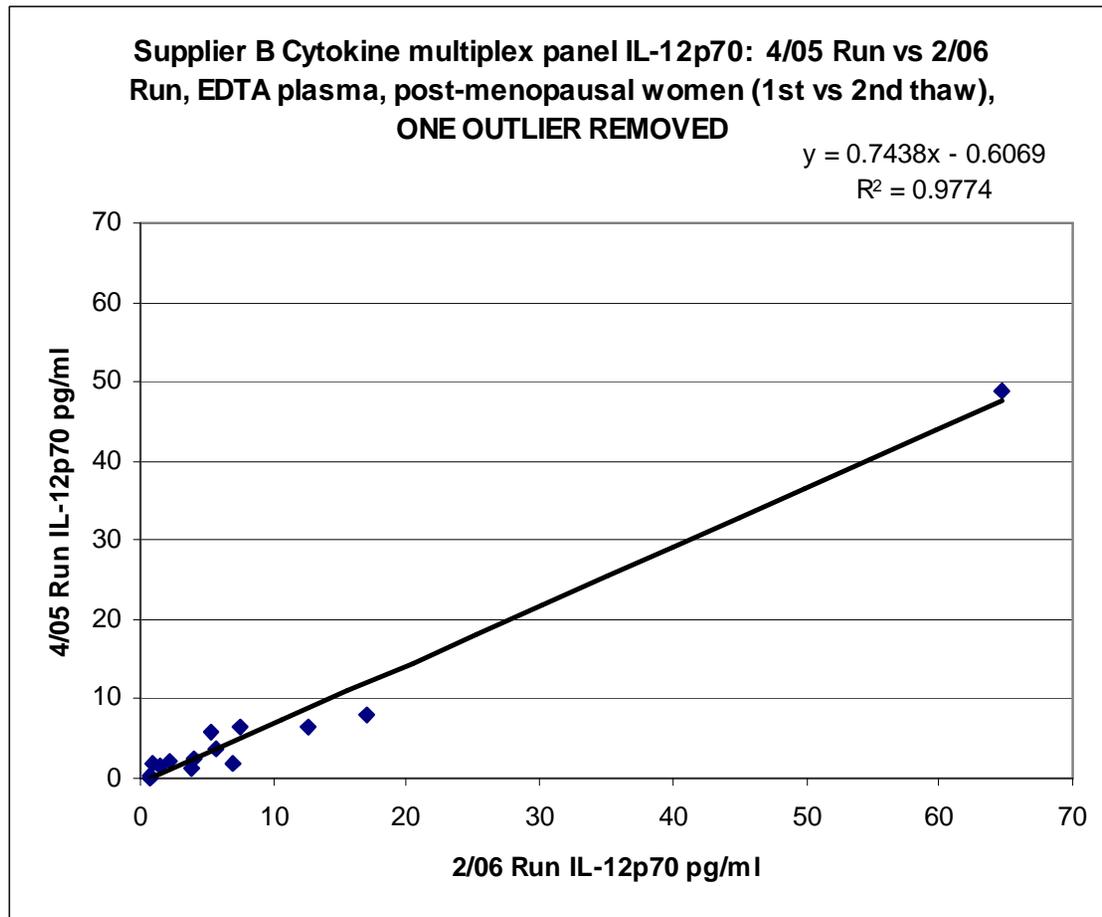
IL-12p70 levels for EDTA plasma samples from a case/control study of post-menopausal women with and without cancer, using Supplier B's Cytokine multiplex panel, assayed 4/05 as a 21-plex, agreed moderately well with levels for the same sample set, assayed 2/06 as a 6-plex, with loss of agreement primarily due to one outlier. Note that a high value such as the outlier here, should be considered spurious in healthy subjects and that a freeze thaw effect may exist but has not been investigated to date.

$R^2 = 0.668$

Supplier B

Human Cytokine multiplex panel

Reproducibility



IL-12p70 levels for EDTA plasma samples from a case/control study of post-menopausal women with and without cancer, using Supplier B's Cytokine multiplex panel, assayed 4/05 as a 21-plex, agreed well with levels for the same sample set, assayed 2/06 as a 6-plex, with one outlier removed.

$R^2 = 0.978$

IL-12p70 summary

- The assays are detecting levels of IL-12p70 in healthy populations, that approach the lower limit of detectability.
- Freeze-thaw effects may exist but have not been investigated formally.
- Very high values should probably be considered spurious in healthy subjects.