IL-1b Validations

- Validation includes:
- % detectability data for Supplier B’s Human CVD3 and 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-1b - % Detectability

• Using supplier B’s Cytokine multiplex panel as a 21-plex, % detectability of IL-1b in serum and plasma from healthy donors:
  • Citrated plasma (n=16):  87.5%
  • Serum (n=20):  20%
• (Note that for each sample type a different set of donors were used.)
Using supplier B’s Human CVD3 panel, % detectability of IL-1b in serum and plasma from healthy donors:

- EDTA plasma (n=10) 90%
- Citrated plasma (n=10) 70%
- Serum (n=10) 50%

(Note that for each sample type a different set of donors were used.)
Supplier B

Human Cytokine multiplex panel

Reproducibility

IL-1b levels for citrated plasma samples from healthy donors assayed with Supplier B’s Cytokine multiplex panel, assayed 8/05 as a 21-plex, agreed moderately well 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.

$R^2 = 0.73$
IL-1b 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 poorly with levels for the same sample set, assayed 2/06 as a 6-plex, primarily due to one outlier. Note that very high values 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.

$R^2 = 0.071$
Supplier B

Human Cytokine multiplex panel

Reproducibility

IL-1β 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 a single outlier removed.

R² = 0.591
Supplier B

Human Cytokine multiplex panel

Reproducibility

IL-1b levels for citrated plasma samples from healthy donors assayed with Supplier B’s Cytokine multiplex panel, assayed 8/05 as a 21-plex, agreed moderately well with levels for the same sample set, assayed 4/06 as a 3-plex. Reproducibility is likely affected by levels that approach the lower limit of detectability.

\[ y = 0.4718x - 0.1469 \]

\[ R^2 = 0.7664 \]
IL-1b summary

• The assays are detecting levels of IL-1b 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.