Fit-for-Purpose Validation of a Soluble CD14 ELISA in Human Plasma: Anomalous Long-Term Stability Pattern for Endogenous Biomarker

**Deborah Martin, Rachel Williams and Masood Khan** - KCAS, Shawnee, Kansas 66216 USA

---

**OBJECTIVE**

The soluble form of CD14 (sCD14) is a potential biomarker of mortality in HIV infection and pneumonia in children, along with being a diagnostic marker for sepsis. In this study, we performed a Fit-for-Purpose validation of a commercial kit-based assay for reliable quantification of sCD14 in human plasma. The validated method was used for initial evaluation of the long-term stability of sCD14 in human plasma samples stored at -80°C.

**INTRODUCTION**

Glycoprotein CD14 is a lipopolysaccharide (LPS) receptor involved in the innate immune system (Fig. 1). Mostly, CD14 exists in the membrane-bound form (mCD14) anchored through glycosylphosphatidylinositol (GPI) on the surface of monocytes, macrophages, dendritic cells and neutrophils. sCD14 can be produced either by secretion or proteolytic cleavage and can be detected in the serum and urine. sCD14 plays a different role in immune-mediated responses than mCD14. The mCD14, in conjunction with the LPS-binding protein (LBP), Toll-like receptor 4 (TLR4) and its co-receptor protein MD2, acts as a receptor for LPS, initiating an immune response. Whereas sCD14 competes with mCD14 for LPS, lowering the anticipated immune response. The sCD14 may also be able to increase the immune response to LPS by mediating LPS-induced activation of cells that do not express mCD14. Therefore, sCD14 may be useful as a potential biomarker in certain disease states. There is evidence for increased levels of sCD14 in the event of sepsis. HIV disease evolution, poly cyclic kidney disease, pediatric lung disease, and rheumatoid arthritis. In this case study, a fit-for-purpose validation of sCD14 assay is described, and analytical method performance characteristics of the assay are presented.

**RESULTS**

**Table 3: Plasma Controls (Precision of Measurements)**

<table>
<thead>
<tr>
<th>Plasma Controls</th>
<th>No. of Runs</th>
<th>No. of Results</th>
<th>sCD14 Conc. (pg/mL)</th>
<th>Precision (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-1</td>
<td>3</td>
<td>7</td>
<td>826032.4 ± 5.1</td>
<td>13.9</td>
</tr>
<tr>
<td>Control-2</td>
<td>3</td>
<td>196699.0 ± 5.3</td>
<td>12.8</td>
<td></td>
</tr>
</tbody>
</table>

Concentrations determined in plasma control samples are used as nominal concentration when these samples are used for stability evaluation experiments.

**Table 4: Method Specificity**

- **LQC and HQC samples** were assayed in the absence (Baseline) and presence of Test Compounds (MCP-1 and MCP-3) at conc. of 600 (in LQC) and 2400 pg/mL (in HQC).

**Table 5: Soluble CD14 Stability in Human Plasma**

**Table 6: Method Selectivity**

- **sCD14 in Human Plasma (pg/mL)**

**Table 7: Dilutional Linearity (Spiked Human Plasma)**

**RESULTS (CONT.)**

**Table 8: Long-Term Storage Stability (Human Plasma)**

- **Typical Stability Criteria:** 67% Stability Sample within 20% of Baseline Concentration.
- **High Controls failed criteria set prior to 113 days and greater.**
- **Low Controls passed the criteria up to 311 days.**
- **Storage time reflects only the time that samples were stored at -80°C after transfer from -20°C freezer (where they were stored for considerably longer time between collection date and analysis).**

**CONCLUSION**

- The modified kit method based for sCD14 quantification in human plasma was successfully validated.
- An anomalous pattern of sCD14 stability (i.e., apparent increase in sCD14 conc. in high controls but not in low controls) was observed. However, stability issue needs further experiments due to limited amount of data.
- Caution should be exercised while analyzing samples for sCD14 after prolonged storage at -20°C / -80°C.

**References**


**Fig. 1:** sCD14: A Diversified Biomarker

**Fig. 2:** Typical Calibration Curve

**Table 1: Range of Quantification (100 – 5,000 pg/mL)**

<table>
<thead>
<tr>
<th>Plasma Controls</th>
<th>No. of Runs</th>
<th>No. of Results</th>
<th>sCD14 Conc. (pg/mL)</th>
<th>Precision (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LQC</td>
<td>3</td>
<td>9</td>
<td>275 ± 2.3</td>
<td>8.8</td>
</tr>
<tr>
<td>HQC</td>
<td>3</td>
<td>9</td>
<td>275 ± 2.3</td>
<td>8.8</td>
</tr>
</tbody>
</table>

**Table 2: Precision & Accuracy**

- **%BL:** % of Baseline Conc. in Freshly Thawed Samples

**Table 4: Method Stability**

- **Typical Stability Criteria:** 67% Stability Sample within 20% of Baseline Concentration.
- **High Controls failed criteria set prior to 113 days and greater.**
- **Low Controls passed the criteria up to 311 days.**
- **Storage time reflects only the time that samples were stored at -80°C after transfer from -20°C freezer (where they were stored for considerably longer time between collection date and analysis).**

**Table 5: Soluble CD14 Stability in Human Plasma**

**Table 6: Method Selectivity**

- **sCD14 in Human Plasma (pg/mL)**

**Table 7: Dilutional Linearity (Spiked Human Plasma)**

**RESULTS (CONT.)**

**Table 8: Long-Term Storage Stability (Human Plasma)**

- **Typical Stability Criteria:** 67% Stability Sample within 20% of Baseline Concentration.
- **High Controls failed criteria set prior to 113 days and greater.**
- **Low Controls passed the criteria up to 311 days.**
- **Storage time reflects only the time that samples were stored at -80°C after transfer from -20°C freezer (where they were stored for considerably longer time between collection date and analysis).**

**CONCLUSION**

- The modified kit method based for sCD14 quantification in human plasma was successfully validated.
- An anomalous pattern of sCD14 stability (i.e., apparent increase in sCD14 conc. in high controls but not in low controls) was observed. However, stability issue needs further experiments due to limited amount of data.
- Caution should be exercised while analyzing samples for sCD14 after prolonged storage at -20°C / -80°C.

**References**