The effects of delayed processing of whole blood samples on immune function and multiplex cytokine assays

Kerri J. Penrose, Troy J. Kemp, Marcus C. Williams, and Ligia A. Pinto
HPV Immunology Laboratory, SAIC-Frederick, NCI Frederick, Frederick MD
Why is standardization of sample collection important?

- Large clinical/epidemiological studies often utilize multiple centers for sample collection.
- Shipping of samples is often required.
- Time between sample collection and processing, among other variables may affect sample quality and results.

What is already established?

- There is a lack of standardization of procedures across different clinical studies.
- A growing body of evidence illustrates the need for standardization and consistency.
- Assays may be differentially affected by variations in procedures.
Primary mission of HPV Immunology Laboratory (SAIC-Frederick):

To investigate immune responses in the context of HPV infection and HPV vaccination.

Investigation of immune responses in clinical studies involves three technical stages:

• Sample collection in a clinical setting
• Processing and cryopreservation
• Testing in batches in the laboratory

*Quality and comparability of cryopreserved samples is critical!
Clinical Studies of Cellular Immunity

**Goal:** To evaluate the effect of processing in PBMC proliferation and cytokine levels in plasma
Clinical Studies of Immune Function

Plasma

Cytokine analysis
(Luminex Technology)

PBMCs

Proliferation
(H³-Thymidine incorporation) +
Cell subset evaluation
(Flow cytometry)
Effects of processing on lymphocyte proliferative responses in PBMCs from healthy individuals
PBMC isolation is accomplished using density gradient centrifugation.

No significant effect on functional lymphoproliferation assays was found in a comparison of different PBMC isolation methods.
Assessing the effect of delayed processing:

Blood collection from patient

0hrs
24hrs

PBMC isolation & Cryopreservation
Delayed processing dramatically reduces proliferative potential of isolated PBMCs from healthy individuals.

**Phytohemagglutinin (PHA)**

- p <= 0.0001

**Poke Weed Mitogen (PWM)**

- p = 0.0001

**Influenza A virus**

- p = 0.004

**HPV 16 L1 Virus-like particles**

- p = 0.002

n=38
Delay in processing affects frequency of immune cell subsets in PBMCs from healthy individuals.

<table>
<thead>
<tr>
<th></th>
<th>0hrs</th>
<th>24hrs</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+ (T cells)</td>
<td>73.9%</td>
<td>62.2%</td>
<td>0.003</td>
</tr>
<tr>
<td>CD19+ (B cells)</td>
<td>9.1%</td>
<td>10.5%</td>
<td>0.226</td>
</tr>
<tr>
<td>CD16/56+ (NK cells)</td>
<td>10.8%</td>
<td>16.5%</td>
<td>0.009</td>
</tr>
<tr>
<td>CD14+ (monocytes)</td>
<td>5.42%</td>
<td>13.4%</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Effects of time of processing on circulating cytokines in Plasma from healthy individuals using multiplex Luminex based technology

<table>
<thead>
<tr>
<th>Cytokine 1</th>
<th>Cytokine 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1a</td>
<td>IL-13</td>
</tr>
<tr>
<td>IL-1b</td>
<td>IL-17</td>
</tr>
<tr>
<td>IL1ra</td>
<td>IL-17</td>
</tr>
<tr>
<td>IL-2</td>
<td>TNF-a</td>
</tr>
<tr>
<td>IL-4</td>
<td>IFN-g</td>
</tr>
<tr>
<td>IL-5</td>
<td>MCP-1</td>
</tr>
<tr>
<td>IL-6</td>
<td>MIP-1a</td>
</tr>
<tr>
<td>IL-8</td>
<td>GM-CSF</td>
</tr>
<tr>
<td>IL-7</td>
<td>G-CSF</td>
</tr>
<tr>
<td>IL-10</td>
<td>Eotaxin</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>EGF</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>VEGF</td>
</tr>
</tbody>
</table>
3 out of 9 detectable cytokines displayed significantly different levels with delayed processing of heparinized plasma.

- **G-CSF**: p = 0.01
- **IL-8**: p = 0.01
- **EGF**: p = 0.04
Summary

• Variations in time between blood collection and sample processing have a significant effect on:
  • Lymphoproliferative responses
  • Lymphocyte cell subsets
  • Size and granularity of cell populations
  • Circulating cytokines

• These results indicate that time between blood collection and processing is a determining factor for a number of immune-related biomarkers.

The establishment and adherence of standardized methods with consistent and minimal times between collection and processing is critical to assure sample quality for immune monitoring studies, involving functional and cytokine profiling assays.
Thank you

SAIC-Frederick, Inc.

HPV Immunology Lab

Ligia A. Pinto, PhD
Troy J. Kemp, PhD
Marcus C. Williams
Yuanji (David) Pan