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

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- **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):

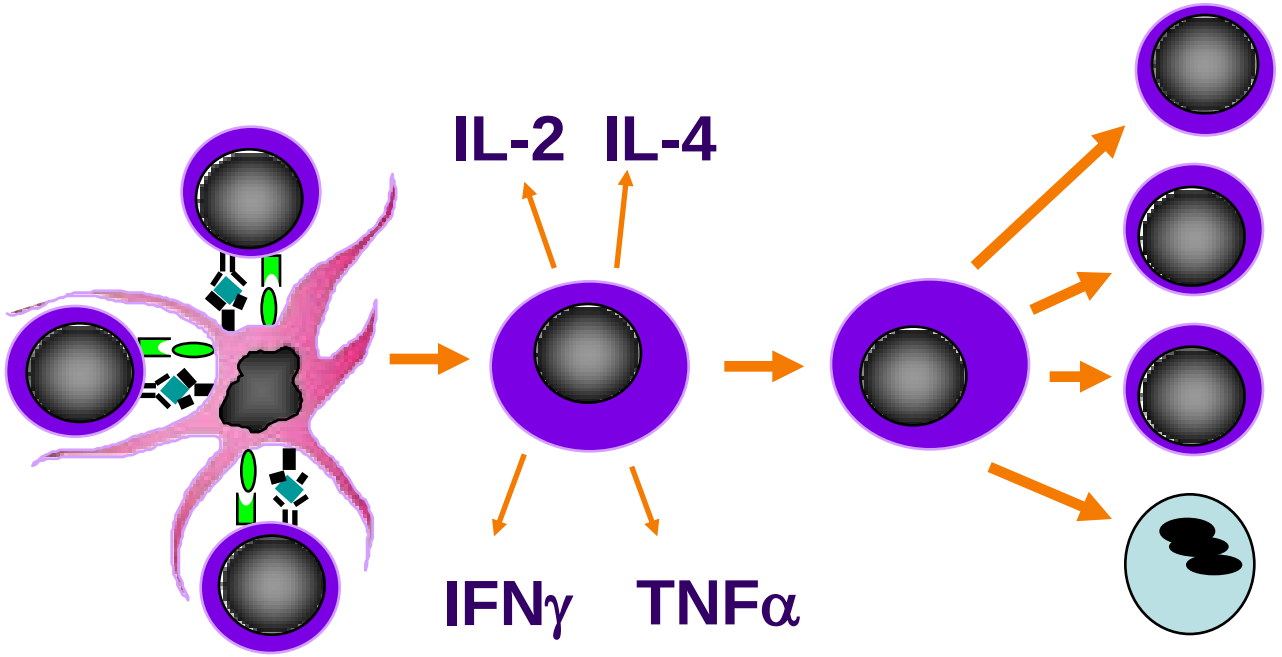
To investigate immune responses in the context of HPV infection and HPV vaccination.

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

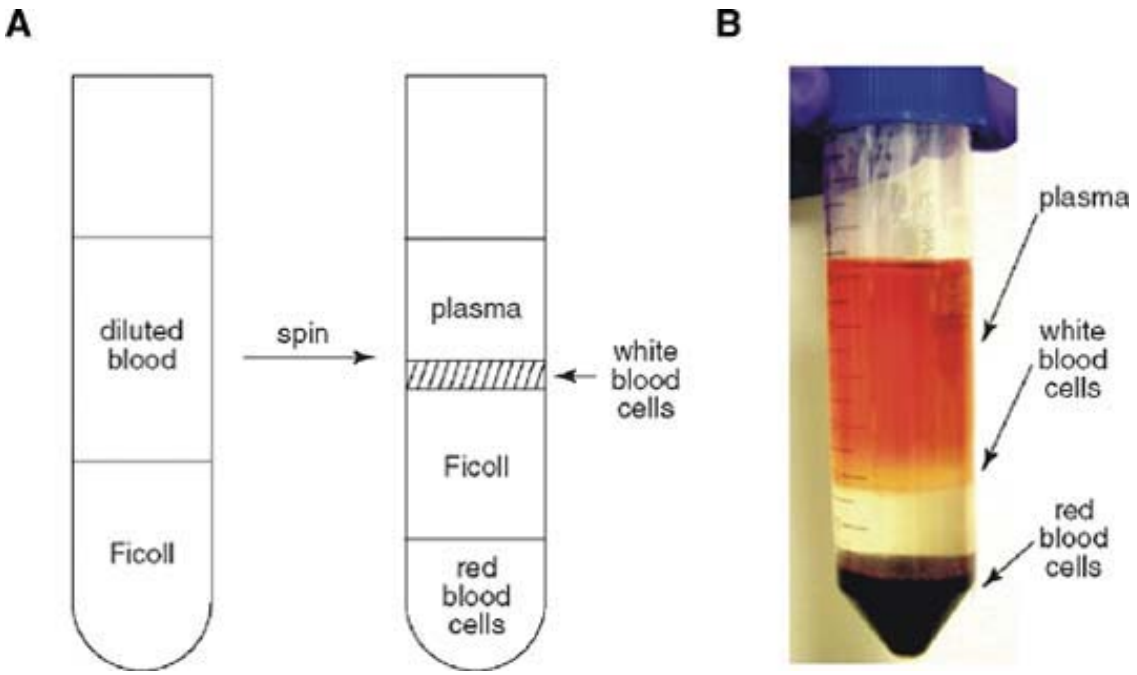
PBMCs

Cytokine analysis
(Luminex Technology)

Proliferation
(H^3-Thymidine incorporation)
+
Cell subset evaluation
(Flow cytometry)

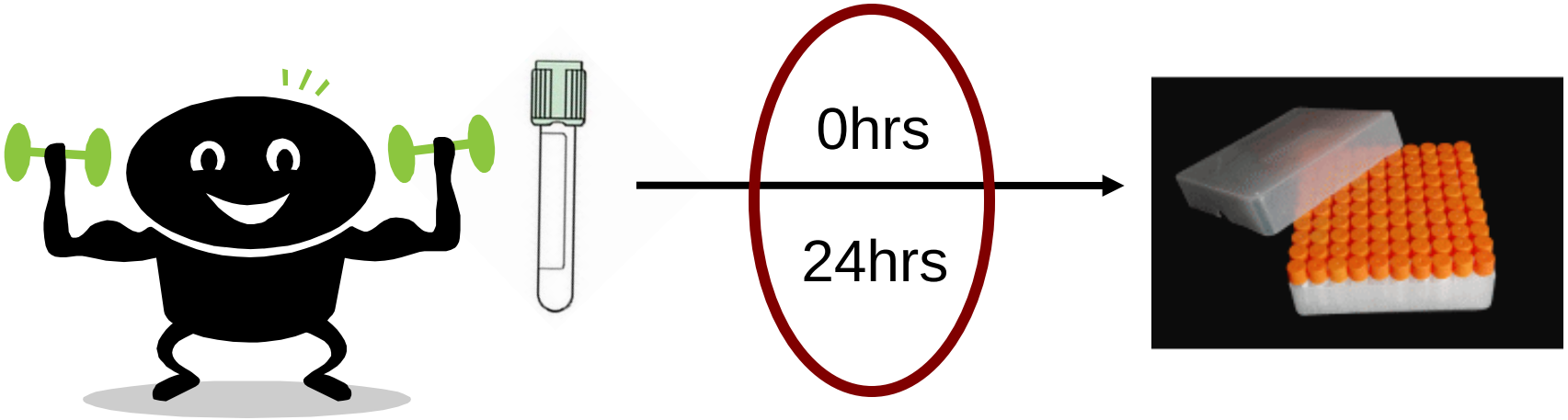
Effects of processing on lymphocyte proliferative responses in PBMCs from healthy individuals

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:

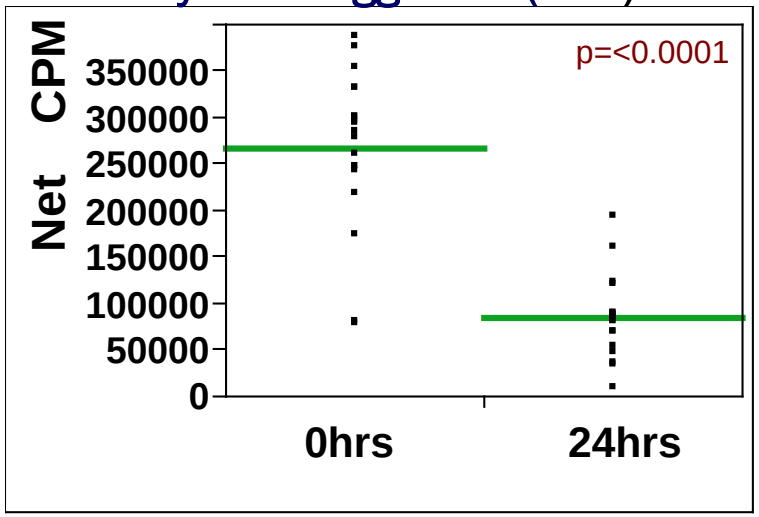


Blood collection from patient

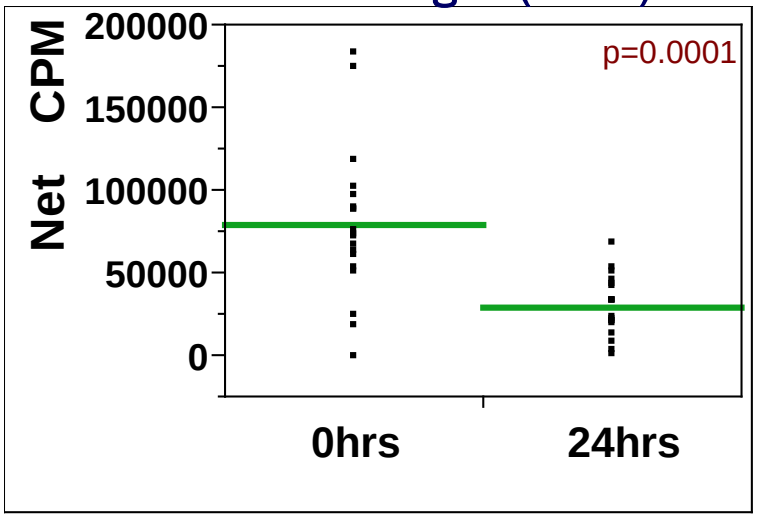
PBMC isolation
&
Cryopreservation

Delayed processing dramatically reduces proliferative potential of isolated PBMCs from healthy individuals

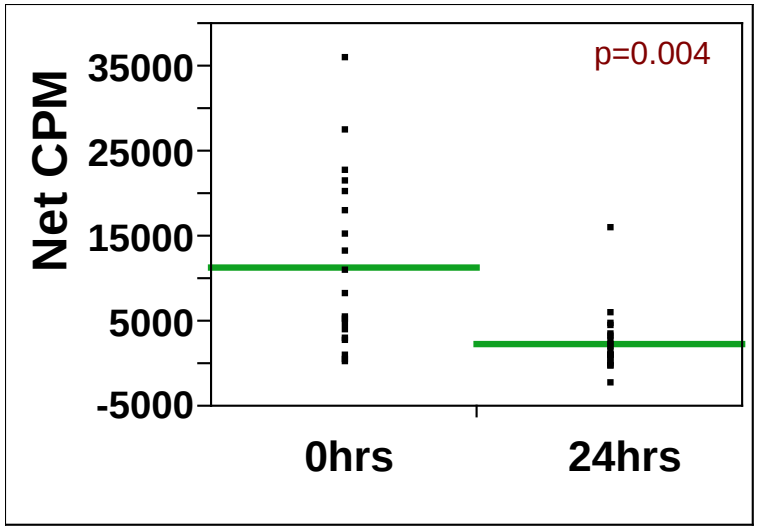
Phytohemagglutinin(PHA)



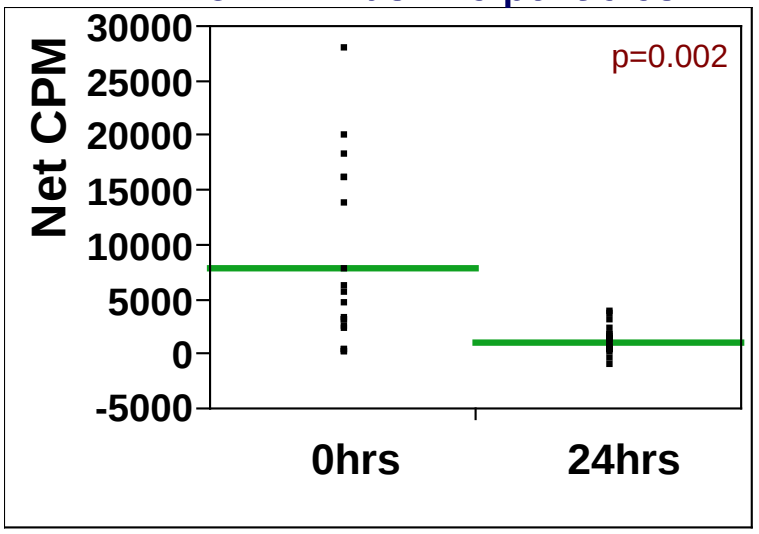
Poke Weed Mitogen (PWM)



Influenza A virus



HPV 16 L1 Virus-like particles

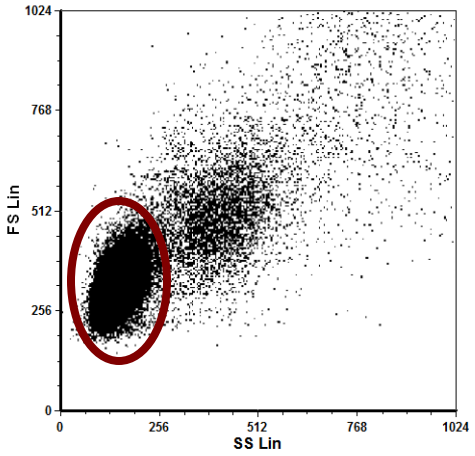


n=38

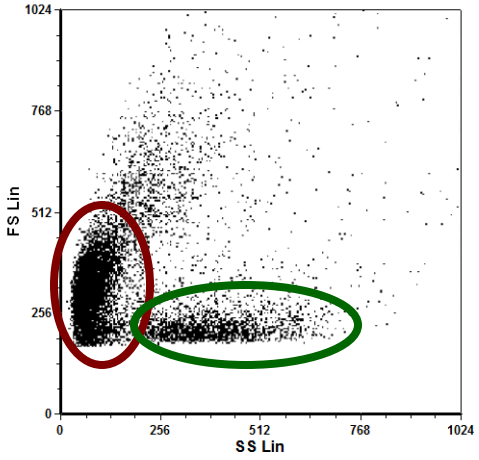
Delay in processing affects frequency of immune cell subsets in PBMCs from healthy individuals

	0hrs	24hrs	p value
CD3+ (T cells)	73.9%	62.2%	0.003
CD19+ (B cells)	9.1%	10.5%	0.226
CD16/56+ (NKcells)	10.8%	16.5%	0.009
CD14+ (monocytes)	5.42%	13.4%	0.002

0hrs



24hrs



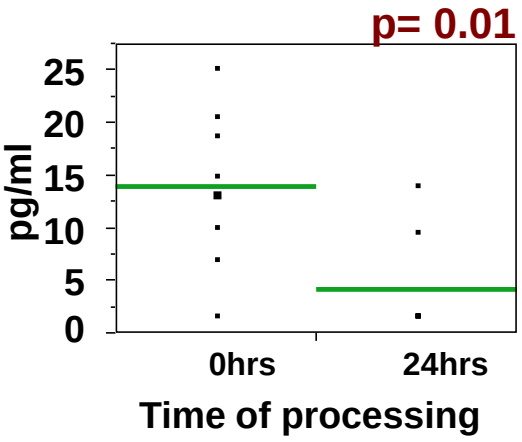
Effects of time of processing on circulating cytokines in Plasma from healthy individuals using multiplex Luminex based technology

IL-1a
IL-1b
IL1ra
IL-2
IL-4
IL-5
IL-6
IL-8
IL-7
IL-10
IL-12p40
IL-12p70

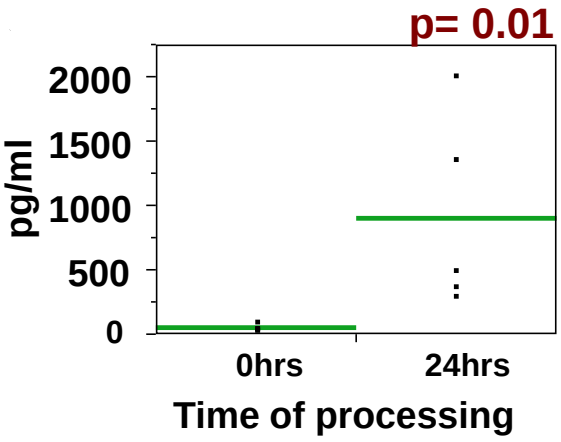
IL-13
IL-17
TNF-a
IFN-g
MCP-1
MIP-1a
GM-CSF
G-CSF
Eotaxin
EGF
VEGF

3 out of 9 detectable cytokines displayed significantly different levels with delayed processing of heparinized plasma.

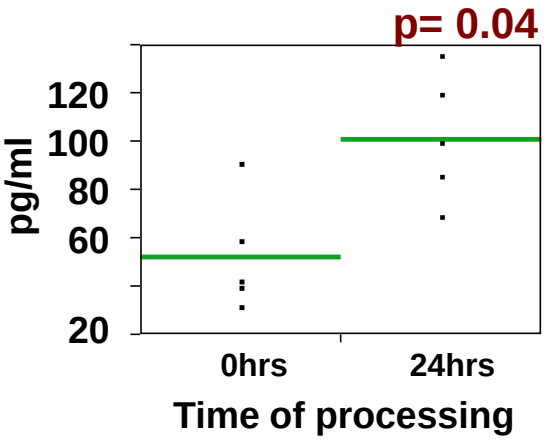
G-CSF



IL-8



EGF



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.

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