# Flu Antigen Recall Responses using TruCulture® with OptiMAP and SIMOA® Analysis

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#### **ABSTRACT**

TruCulture® is a whole blood collection and culture system that is designed to be used at clinical sites to investigate human immune responses under specific stimulation conditions. OptiMAP is a Multi-Analyte Profile (MAP) bead based immunoassay panel that measures 13 analytes that span the major immune responses (T\_1, T\_2, T\_17, neutrophil, and monocyte/macrophage activation). The recall response of flu hemagglutinin (HA) antigens was examined in healthy adults vaccinated with the 2018-2019 annual flu vaccine. Whole blood was drawn into TruCulture tubes: null (media only), staphylococcal enterotoxin B (SEB), and null tubes spiked with 1.25mg each of 4 different recombinant HA proteins. All samples were incubated for 48 hours at 37°C. Supernatant were collected and analyzed using the OptiMAP panel. No inflammatory cytokines were detected in the null tubes from either the baseline or 2 weeks post-vaccination samples. There were no differences in cytokine production in the SEB stimulated samples between the baseline and 2 weeks post-flu vaccination time lines. For flu HA stimulated samples, there was a significant increase in production of GM-CSF, IFN-γ, IL-1β, IL-6, IL-12p70, IL-23, and TNF-α at 2 weeks post-flu vaccination compared to samples collected at baseline. Additionally, flu HA stimulated samples post vaccination also demonstrated increased production of type I inteferons, as analyzed by SIMOA. These data demonstrate that TruCulture in conjunction with OptiMAP and SIMOA are useful tools for investigating antigen specific responses.

### MATERIALS AND METHODS

#### **TruCulture Tubes**

- Null 782-001086
- S.aureus enterotoxin type B (SEB) 782-001124
- Null + Flu Antigens (5µg/tube)

### Flu Antigens (Immune Technology Corp.)

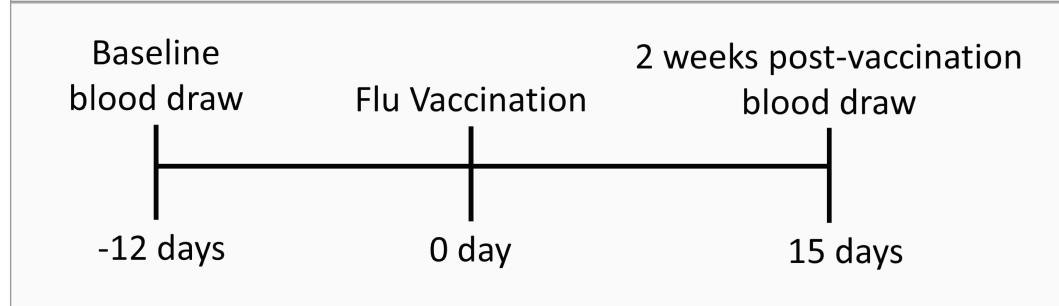
Recombinant proteins are at 1mg/mL

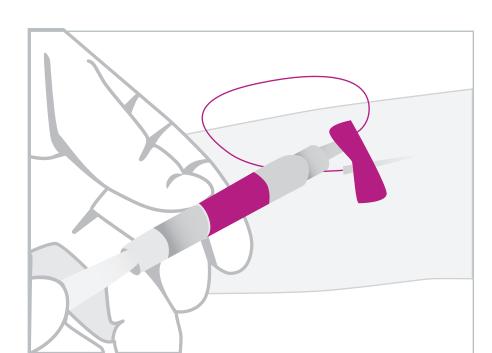
- HA1(H3N2)(A/Singapore/INFIMH-16-0019/2016)
- HA2(B/Colorado/06-2017)
- HA1(B/Phuket/3073/2013)
- HA1(H1N1)(A/Michigan/45/2015)

#### TNF-a IFN-γ **ENA-78** IL-12p70 IL-6 IL-2 IL-8 **IL-10 IL-1**β **IL-17 IL-23**

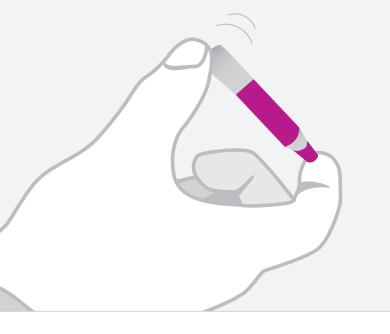
#### **IL-13 GM-CSF Experimental Protocol:**

**OptiMAP – 13 Analyte Multiplex Assay** 





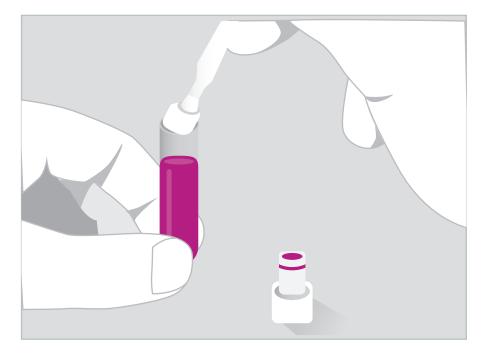
01. COLLECT Draw 1 mL of blood directly into the TruCulture Tube and break off the plunger.



02. MIX Gently invert tube to mix 3 to 5 times



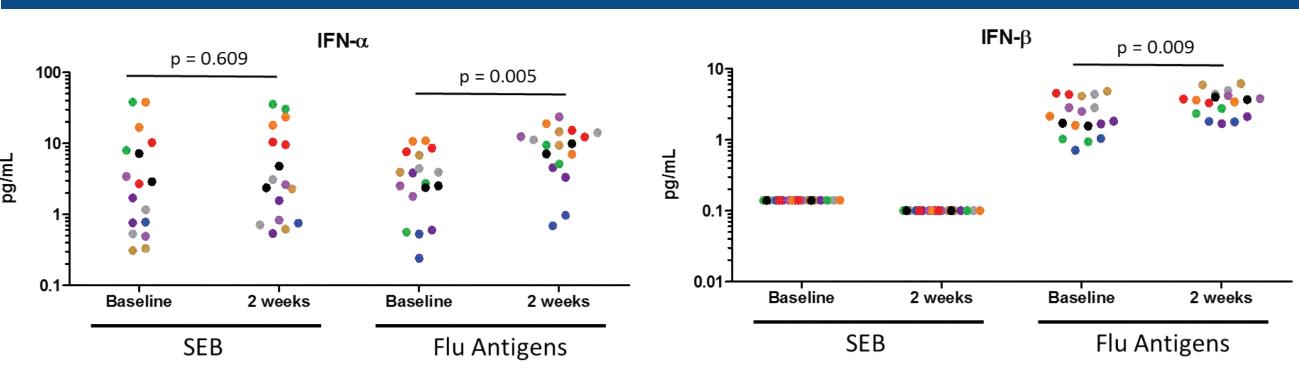
03. INCUBATE Place tube in 37°C heat block for up to 24 or 48 hours.



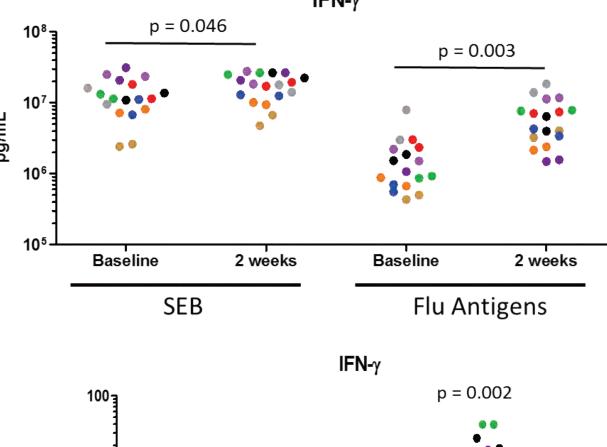
04. SEPARATE Manually insert valve to separate supernatant from the cells. Collect supernatant and cell layer for downstream analysis.

# **RESULTS**

# Flu Vaccination Increased Type I and Type II Interferons as Analyzed by SIMOA



SIMOA Analysis of TruCulture Supernatants: Supernatants from TruCulture samples were analyzed by SIMOA. Each tube is plotted as a separate dot, duplicate tubes from the same subject are represented by the same color. Analysis was done by paired Student's T test. Increased production of type I and type II interferons during antigen recall suggests a stronger anti-viral response post-vaccination. Additionally, the vaccinated group showed a basal level of increased IFNy most likely due to vaccine induced T cell activation.



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#### **SUMMARY**

- TruCulture is an easy to use culture system to investigate immune responses during antigen recall after vaccination.
- UltraSensitive SIMOA assays can be used to detect changes in analytes at low concentrations.
- Relative IFNy concentrations correlated between OptiMAP and SIMOA.

#### TRUCULTURE A Blood Collection and Whole Blood Culture System

#### TruCulture whole blood culture

- Integrated closed sterile whole blood collection and culture system
- Retains all blood components, including all leukocytes, platelets, soluble factors, and Fc receptor expressing cells
- Easy to use, eliminates the need for cell manipulation
- Standardized to ensure consistent performance across multiple users and clinical sites

#### PBMC (Peripheral blood mononuclear cells) culture

- Separate blood collection and specialized cell culture procedures
- Does not retain all blood components, including granulocytes (ex. neutrophils which makes up >50% of all circulating leukocytes), soluble factors, etc.
- Extensive manipulation, processing, and often freezing/shipping prior to culturing
- Poor reproducibility



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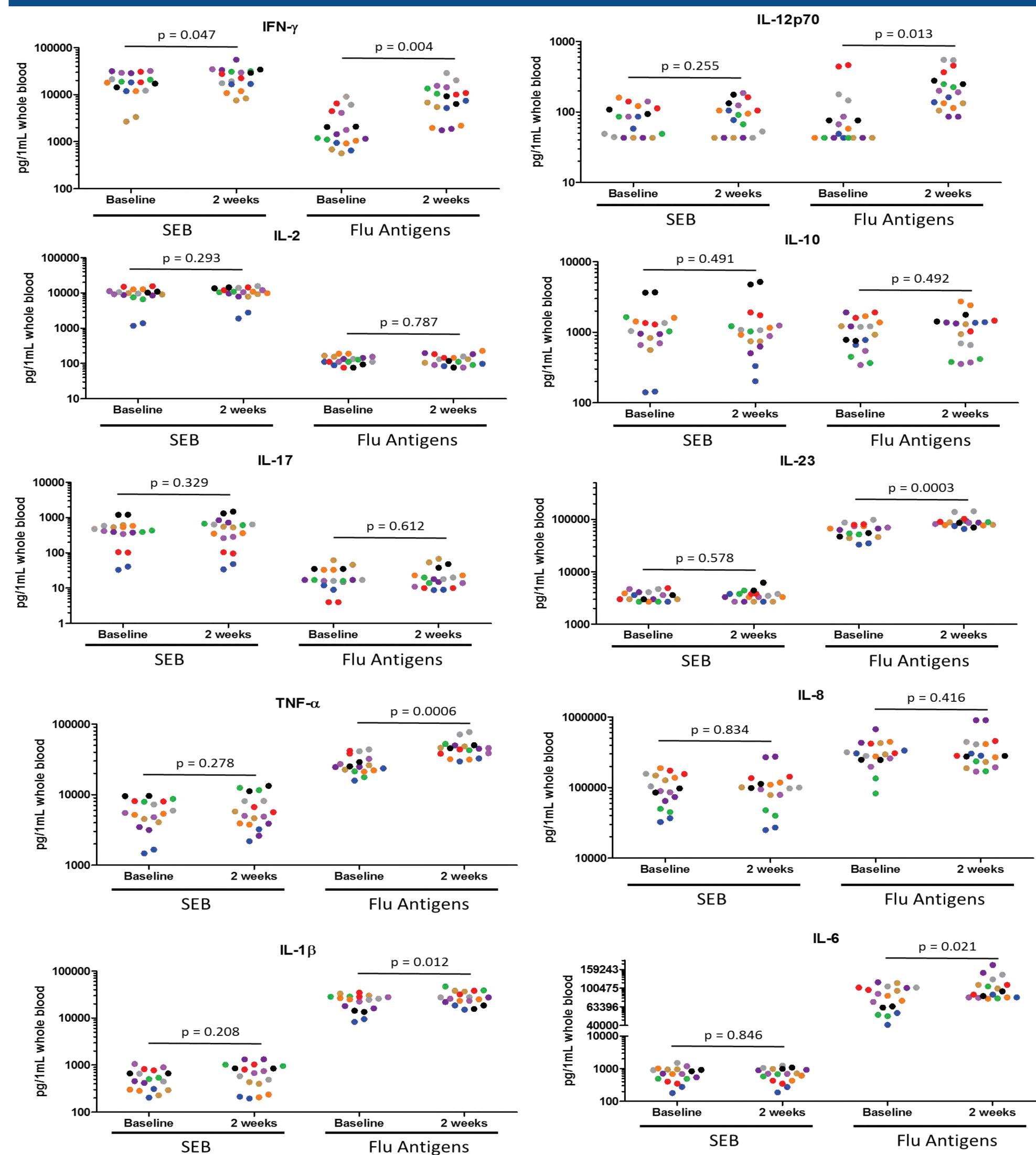


**Proprietary Culture Medium** (allows incubations up to 48 hrs.)

**Rubber Seal** (closed system, before and after blood draw, reduces risk of sample contamination)

# **RESULTS**

#### Increased Inflammatory Responses to Flu Antigens Post-Vaccination by OptiMAP



OptiMAP Analysis of TruCulture Stimulated Samples: Supernatants from TruCulture samples were analyzed by OptiMAP. Each tube is plotted as a separate dot, duplicate tubes from the same subject are represented by the same color. Analysis was done by paired Student's T test. Whole blood stimulated with Flu Antigens after 2 weeks vaccination increased production of protective adaptive immune responses, such as IFNy, IL-12p70, and IL-23. Additionally, increased proinflammatory cytokine production were also observed. SEB stimulated samples did not demonstrate the same recall response after vaccination.