

## Ex-Vivo Cytokine Stimulation

**Test Name:** Ex-Vivo Cytokine Stimulation

**Background:** Ex-vivo stimulation is a powerful method to assess cellular responses to various external stimuli, including antigens, mitogens, bacterial products, etc. In the IBT test format, heparinized human whole blood is incubated with stimulant. Following this incubation, the supernates are assayed for the concentration of cytokines released. The method can be used to assess cellular immune status (activation or suppression) and to investigate the effect of pharmacologic agents on cellular processes. A number of different cytokines can be tested with this approach. Many of these have been classified by investigators according to function (e.g. Th1, Th2, etc.).

As the use of cytokine and anticytokine therapies and other immunoregulatory treatments increase, monitoring cytokine changes in cellular secretion will become increasingly important. In some cases, the measurement of a panel of cytokines may be an effective marker for the clinical effects of novel therapeutics for which no single marker provides sufficient information.

**Test Applications:** The testing is customized for each client's specific research project, including the stimulation conditions and the cytokines to be measured. Some examples of Ex-Vivo research applications include the following:

- Quantify CMI to microbial antigens
- Immunotoxicity studies
- Enhancing & Inhibiting effects of drugs on responses to stimulants such as LPS.
- Drug or chemical stimulation of cytokine release

### Steps in the Testing Process:

**(1) Cell Stimulation.** Heparinized whole blood is incubated with a stimulant. The doses and the incubation times (usually in the 1 to 24 hour range) depend on the stimulant and the cytokines to be measured. PHA and RPMI cell culture medium alone are generally used as positive and negative controls, respectively. Following the stimulation, plasma is collected and stored at  $-70^{\circ}\text{C}$  until the cytokines are measured.

**(2) Cytokine Measurement.** These are measured with a multiplex array format with the Meso Scale Discovery Sector Imager 2400. MSD cytokine assays measure from one to ten cytokines in a 96-well MULTI- SPOT plate. The assay employs a sandwich immunoassay format. MSD technology uses electrochemiluminescence detection; a CCD camera allows for the quantification of light emitted from each spot in each well. MSD software generates a standard curve to determine sample cytokine concentrations.

**Stimulants:** The following are stimulants that can be used with this Ex-Vivo method:

- Mitogens (e.g. PHA)
- Lipopolysaccharide (LPS)
- Bacterial, Fungal and Viral Protein Antigens
- Haptens and Conjugates (Drugs, Chemicals, etc)

### Cytokines Currently Available for Ex-Vivo Testing: Panels:

- Th1/Th2 Panel (Serum) or (Plasma)
- Pro-Inflammatory Panel (Serum) or (Plasma)
- Custom Panels available upon request

### Individual Cytokines:

- Interleukin-1 Beta (IL-1 $\beta$ )
- Interleukin-2 (IL-2)
- Interleukin-4 (IL-4)
- Interleukin-5 (IL-5)
- Interleukin-6 (IL-6)
- Interleukin-8 (IL-8)
- Interleukin-10 (IL-10)
- Interleukin-12 (IL-12p70)
- Interleukin-13 (IL-13)
- Interferon-gamma (IFN- $\gamma$ )
- Granulocyte- Macrophage- CSF
- TNF-alpha (TNF- $\alpha$ )

(AVAILABLE FOR CONTRACT RESEARCH ONLY)

**Specimen:** Volume of blood required will depend on the number of stimulants. Call for information.

**Handling and Transport of Specimens:** Specimens should be shipped via overnight courier at ambient temperatures. Avoid extreme temperatures. The specimen must be received within 24 hours of the blood draw.

### References:

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- Mazurek GH, et al. Comparison of a whole-blood IFN- $\gamma$  assay with tuberculin skin testing for detecting latex Mycobacterium tuberculosis infection. *JAMA* 2001; 286:1740-1747.
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- House RV. Cytokine measurement techniques for assessing hypersensitivity. *Toxicology* 2001; 158: 51-58.
- Bienvenu JA et al. Cytokine assays in human sera and tissues. *Toxicology* 1998; 129:: 55-61.

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