

## **TEST: T<sub>H</sub>1/T<sub>H</sub>2 CYTOKINES RATIOS BY FLOW CYTOMETRY**

### **PRINCIPLE**

The ratios of T<sub>H</sub>1/T<sub>H</sub>2 cytokines in the cytoplasm of CD3<sup>+</sup>CD4<sup>+</sup> lymphocytes can be determined by flow cytometry. Lymphocytes (mononuclear cells) are stimulated with phorbolmyristic acid (PMA) and ionomycin in the presence of Golgiplug (brefeldin A) (a Golgi transport inhibitor) for 18 hrs, harvested, permeabilized and then reacted with phycoerythrin-anti-cytokine antibodies to TNF- $\alpha$ , or IFN- $\gamma$  or IL-10. Four color flow cytometry is used to identify the percentages of intracellular cytokines in CD3<sup>+</sup>CD4<sup>+</sup> lymphocytes. Stimulation causes the down-regulation of CD4 molecules on the surface of cells and prevents their positive identification. Therefore, CD3<sup>+</sup>CD4<sup>+</sup> cells are identified by negative gating using ECD-anti-CD3 and FITC-anti-CD8. The lymphocytes that do not bind to CD3 and CD8 (CD3<sup>+</sup>CD8<sup>-</sup>) are the CD3<sup>+</sup>CD4<sup>+</sup> cells. The percentages of intracellular cytokine containing CD3<sup>+</sup>CD8<sup>-</sup> cells are determined for each of the three cytokines and then the ratios calculated for TNF- $\alpha$ /IL-10 and IFN- $\gamma$ /IL-10. Women with recurrent spontaneous abortions may have higher ratios than women with normal pregnancy history.

### **SPECIMEN REQUIREMENTS:**

**30-40 ml of whole blood collected in green top tubes with heparin.** Make sure the blood is mixed well after it is drawn from the patient to prevent clots. Send blood at room temperature. **Do not refrigerate.** Deliver to the laboratory within 24 hours. Criteria for an unacceptable sample are a cold specimen (due to refrigeration or shipment on ice), extensive clotting or hemolysis or specimens more than 48 hours old. If specimens are more than 48 hours old, the lymphocytes will be isolated from the blood specimens. Viability of the cells will be determined. If the viability is greater than 80%, the assay will be performed. If the viability is less than 80%, the specimens will be rejected.

### **METHOD: Flow Cytometry**

### **REFERENCES**

1. Ng SC, Thaker P, Gilman-Sachs A, Beaman KD, Beer AE, Kwak-Kim JYH. 2002 Expression of Intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. Am J Reprod Immunol 48:77-86
2. Sacks GP, Clover LM, Bainbridge DR, Redman CW, Sargent IL, 2001 Flow cytometric measurement of intracellular Th1 and Th2 cytokine production by human villous and extravillous cytotrophoblast. Placenta 22:550-9
3. Prussin C, Metcalfe DD. 1995 Detection of intracytoplasmic cytokine using flow cytometry and directly conjugated anti-cytokine antibodies. J Immunol Method. 188:117-28
4. Schuerweigh, A.J., Stevens, W.J. Evaluation of monensin and brefeldin A for flow cytometric determination of interleukin-1 beta., interleukin-6, and tumor necrosis factor-alpha in monocytes. Cytometry 46:172-176-2001.

**Normal Range: TNF- $\alpha$ /IL10 (CD3<sup>+</sup>CD4<sup>+</sup>): 13.2 – 30.6**

**IFN- $\gamma$ /IL10 (CD3<sup>+</sup>CD4<sup>+</sup>): 5.8 – 20.5**

**Turnaround Time: 3 days**