**TEST: TH1:TH2 CYTOKINE RATIO BY FLOW CYTOMETRY WITH PREDNISOLONE**

**PRINCIPLE:**
The ratio of TH1:TH2 cytokines in the cytoplasm of CD3+CD4+ 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) and Prednisolone (a human corticosteroid that inhibits inflammatory cell activation) for 18 hrs. Cells are then harvested, permeabilized and then reacted with phycoerythrin-conjugated antibodies to TNF-$\alpha$, IFN-$\gamma$, or IL-10. Four color flow cytometry is used to identify the percentages of intracellular cytokines in CD3+CD4+ lymphocytes. Stimulation causes the down-regulation of CD4 molecules on the surface of cells and prevents their positive identification. Therefore, CD3+CD4+ 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+CD8-) are the CD3+CD4+ cells. The percentages of intracellular cytokine containing CD3+CD8- cells are determined for each of the three cytokines and then the ratios calculated for TNF-$\alpha$:IL-10 and IFN-$\gamma$:IL-10.

Results from this test are compared to results from the TH1:TH2 Intracellular Ratio test without Prednisolone, to assess the ability of Prednisolone to inhibit CD3+CD4+ lymphocyte activation.

**SPECIMEN REQUIREMENTS:**
30-40 ml of whole blood collected in green top tubes with heparin. Make sure 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 a specimen is more than 48 hours old, the lymphocytes will be isolated and viability of the cells will be determined. If viability is greater than 80%, the assay will be performed. If viability is less than 80%, the specimen will be rejected.

**NOTE:** The TH1:TH2 Intracellular Cytokine Ratio with Prednisolone may **only** be performed in conjunction with the TH1:TH2 Intracellular Cytokine Ratio test.

**METHOD:** Flow Cytometry

**REFERENCES**

**Turnaround Time:** 3 days