

## **TEST: FUNCTIONAL NATURAL KILLER ASSAY**

### **PRINCIPLE:**

Natural killer cells (NK) (large granular lymphocytes) are mononuclear cells that mediate cytolysis against cells that lack MHC Class I or Class II molecules; they also regulate the immune system, mediate natural resistance against tumor cells and produce cytokines. Mononuclear cells (containing lymphocytes with natural kill cell activity) are incubated for 2 hours with target K562 cells that have been pre-stained with PKH-67, a lipophilic dye that binds to the cell membrane and fluoresce green. If K562 cells are killed by NK cells, they become permeabilized, admit propidium iodide dye and fluoresce bright red/orange. Percentage of killed target cells that contain propidium iodide can then be measured by flow cytometry. When lymphocytes are incubated with IVIg and target cells, inhibition of cytotoxicity may be seen.

### **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. Trinchieri, G. Biology of Natural Killer Cells. Adv. Immunol. 47:187-376;1989
2. Starkey, P.M., Sargent, I.L., Redman, C.W.G. Cell population in human early pregnant decidua: Characterization and isolation of large granular lymphocytes by flow cytometry. Immunology 65:129;1988
3. Gilman-Sachs, A., DuChateau., B.K., Aslakson, C.J., Wohlgemuth, G.P., Kwak, J.Y., Beer, A.E., and Beaman, K.D., 1999. Natural Killer (NK) cell subsets and NK cell cytotoxicity in women with histories of recurrent spontaneous abortions. Am. J. Repro. Immunol. 41: 99-105

### **Normal Range for NK Assay Full Panel (Note: NK Assay follow-up does not include the IVIg Concentrations)**

#### NK Assay

% killed	Normal Range
50:1	10 - 40%
25:1	5 - 30%
12.5:1	3 - 20%

#### IVIg Concentrations

12.5 mg	50:1	>30%
	25:1	Inhibition of NK
6.25 mg	50:1	>30%
	25:1	Inhibition of NK

#### Includes Immunophenotype Panel

CD3, CD56, CD19 & CD19/CD5

**Turnaround Time:** 3 days