



### TEST: FUNCTIONAL NATURAL KILLER ASSAYS

#### PRINCIPLE:

Natural killer cells (NK) (large granular lymphocytes) are mononuclear cells that mediate cytotoxicity 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 prestained with PKH-2, a lipophilic dye that binds to the cell membrane and fluoresce green. If K562 cells are killed by NK cells, they become permeabilized and 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 target cells as well as IVIg or Intralipid, inhibition of cytotoxicity may be seen.

#### 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.

#### 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

#### NK Assays Offered:

NK Assay Full Panel	NK Cell Inhibition Panel	NK Assay Follow-up	
% Killed at:	% Killed at:	% Killed at:	Normal Range
50:1	50:1	50:1	10 - 40%
25:1	25:1	25:1	5 - 30%
12.5:1	12.5:1		3 - 20%
IgG 12.5 mg/ml, 50:1	IgG 12.5 mg/ml, 50:1		
IgG 12.5 mg/ml, 25:1	IgG 12.5 mg/ml, 25:1		
IgG 6.25 mg/ml, 50:1	Intralipid 1.5mg/ml, 50:1		
IgG 6.25mg/ml, 25:1	Intralipid 1.5mg/ml, 25:1		

(Note: NK Assay Follow-up does not include IVIg or Intralipid)

Includes Immunophenotype Panel:

CD3+ (T cells), CD56+CD3- (NK cells), CD19+ (B cells), & CD19/CD5

**Turnaround Time:** 3 days