



TEST: LEUKOCYTE ANTIBODY DETECTION

PRINCIPLE:

The cross-match test is an *in vitro* test to determine the presence of anti-lymphocyte antibody to donor cell antigens (lymphocytotoxic antibody) in serum of an individual with preformed antibodies to donor cells. Examples are recipients for an organ transplant or a couple with a history of recurrent spontaneous abortions. The recipient serum is incubated with donor lymphocytes and the binding can be detected by flow cytometry analysis (with fluorescent conjugated reagent). If cytotoxic antibodies are present in maternal serum, they will combine with the surface antigens of donor lymphocytes; the amount of fluorescence on the cells (percentage of positive T or B cells), as measured by flow cytometry, is proportional to the amount of antibody (flow cytometry cross-match).

SPECIMEN REQUIREMENTS:

- **30-40 ml of whole blood collected in green top tubes with heparin from donor (male).**
- **one 10ml red top tube with no additive, or serum separator tube (gel barrier), from recipient (female).**

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. **(Male)** 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. **(Female)** Stability for serum is 24 Hours Room Temperature, 7 days Refrigerated, and -80 Frozen Indefinitely.

METHOD: Flow Cytometry.

REFERENCES:

1. Cook, D.J., *et al.* Transplant Proc. 20: Suppl. 1:81, 1988.
2. Gilman-Sachs, A., Sung-Ping, L., Beer, A.E., and Beaman, K.D. J. Clin. Lab. Immunol. 30, 53-59, 1989.
3. Inhibition of binding of anti-CD3 antibodies to paternal lymphocytes correlates with failure to immunotherapy for treatment of recurrent spontaneous abortions. Gilman-Sachs, A., Harris, D., Beer, A.E., and Beaman, K.D. J. Reprod. Immunol. 17, 41-51, 1990.

Normal Range: Negative

For specimens positive by flow cytometry, % positive T or B cells will be reported for IgM or IgG antibodies.