

T Cell Function Testing (T Cell Fx™)

Test Names: T Cell Fx™- Select and T Cell Fx™- Complete

Alternate Names:

T Cell Energy Test
T Cell Function Test
Lymphocyte Activation/Proliferation Test
Mitogen/Antigen Response Test

Panel Test Code: 403065 T Cell Fx™- Select
403066 T Cell Fx™- Complete

CPT Codes: T Cell Fx™- Select 86353 x 2
T Cell Fx™- Complete 86353 x 7

Background: T lymphocytes are responsible for cell-mediated immunity and are essential for the development and regulation of antibody responses. They produce cytokines in response to stimulation with mitogens or with previously encountered specific antigens. CD69 is expressed upon activation of the T cell with a specific antigen or mitogen; detection of this marker in conjunction with cell surface markers and intracellular cytokines allows detection of specific T cell activation. CD69 expression closely parallels the dose-response curve observed for ³H thymidine incorporation. This test includes culture of peripheral blood mononuclear cells with stimuli listed below, with an agent to block release of newly synthesized cytokines, followed by staining of the extracellular markers and intracellular interferon-γ (IFNγ). The detection of cells in this population is a quantitative indication of the immune response to the antigen used, and may be altered in primary T cell immunodeficiency or immunosuppression.

Tests included:

T Cell Fx™ – Select:

Unstimulated, Phytohemagglutinin (PHA), and Viral Mix (VZV, CMV, Mumps)

T Cell Fx™ – Complete:

Unstimulated, PHA, Viral Mix, Viral Peptide Mix (CMV, EBV, Flu), Tetanus toxoid, *Candida albicans*, *Staphylococcus enterotoxin B* (SEB), and Pokeweed Mitogen (PWM)

Method:

Peripheral blood mononuclear cells are stimulated with mitogens or antigens or left unstimulated as a negative control and incubated at 37°C. During the incubation Brefeldin A is added, causing the interferon-γ to be retained inside the cell. Following the stimulation phase, cells are recovered, stained for surface markers and intracellular IFNγ, and analyzed by flow cytometry.

Specimen Requirements:

8 mL of sodium heparin-anticoagulated blood is required. Blood should be collected and mixed well to avoid clotting. Do not use lithium heparin, ACD, or EDTA as anti-coagulant. Due to the time constraints of the assay, samples need to be collected between the hours of 12pm-5pm Central Time Monday-Thursday. The specimen must be shipped by **priority overnight** courier at ambient temperature. Temperature extremes must be avoided, and the test must be performed within 24 hours of collection.

Units and Normal Reference Range:

The reported result is the percent of T cells that have upregulated CD69 and produced IFN-γ in response to stimulation (X 100). The reference ranges for recall antigens are based upon power analysis for significance. For mitogens the reference ranges are the lower limits of the 95th percent confidence interval.

Turn-around time: 2-3 days.

Clinical Utility & Interpretative Comments:

The test measures the T cell response to antigens of common infections as well as to mitogens, allowing detection of deficiencies in this arm of the immune response. Patients with primary immunodeficiency may have deficits in the acquired T cell response. It is useful for the following types of patients:

- ◆ Immune deficiency
- ◆ Recurrent infection
- ◆ Evaluation of cell-mediated immunity
- ◆ Monitoring immunosuppression (e.g., transplantation patients)

Selected References:

1. Maecker, HT, VC Maino, and LJ Picker. 2000. Immunofluorescence analysis of T-cell responses in health and disease. *J Clin Immunol* 20:391. (Review)
2. Lamoreaux, L, M Roederer, and R Koup. 2006. Intracellular cytokine optimization and standard operating procedure. *Nature Protocols* 1:1507.
3. Maino, VC, MA Suni, JJ Ruitenberg, and RM Smith-McCollum. FastImmune Assay System. BD Biosciences.
4. Betts, MR, MC Nason, SM West, et al. 2006. HIV non-progressors preferentially maintain highly functional HIV-specific CD3+ T cells. *Blood* 107:4781.
5. Horton, H, EP Thomas, JA Stucky, et al. 2007. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. *J. Immunol. Methods* 323:39.
6. Suni, MA, HS Dunn, PL Orr, et al. 2003. Performance of plate-based cytokine flow cytometry with automated data analysis. *BMC Immunol.* 4:9.
7. NCCLS. Performance of Single Cell Immune Response Assays: Approved Guideline. NCCLS document I/LA26-A. Vol. 24, No. 29.
8. Herzenberg LA, J Tung, WA Moore, et al. 2006. Interpreting flow cytometry data: a guide for the perplexed. *Nature Immunol.* 7:681.
9. Simms PE and TM Ellis. 1996. Utility of flow cytometric detection of CD69 expression as a rapid method for determining poly- and oligoclonal lymphocyte activation. *Clin Diag Lab Immunol* 3:301.

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