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Introduction

The purpose of this booklet is to inform physicians of the commonly used flow cytometric tests for the evaluation of primary immunodeficiency disorders (PIDDs). PIDDs can present at any age and are characterized by recurrent infections, severe infections requiring hospitalization or intravenous antibiotics, and infections caused by opportunistic or unusual organisms. The ability to characterize and define these disorders has improved greatly as our understanding of immunology has progressed.

The evaluation of PIDDs has benefited from the use of flow cytometry. Flow cytometry utilizes antibodies or reagents that emit fluorescence to enumerate the subsets of peripheral blood leukocytes and characterize the functional capacity of these cells. By staining peripheral blood leukocytes with antibodies that are specific for defined antigens, detailed assessments of the different components of the immune system are possible. This educational booklet will provide a brief overview of PIDDs and the specific flow cytometric tests that can be used to diagnose these disorders.

Introduction to flow cytometry

Flow cytometry is a technique in which fluorescently labeled cells flow through a cytometer a single cell at a time. The fluorescent compounds are excited with a laser and detectors measure the light emitted from these compounds. Different fluorescent compounds emit light at different wavelengths, which allows for the discrimination of several different proteins on a cell. When multiple fluorescent compounds are used to analyze a cell population, the results typically are depicted in two-dimensional diagrams. For example, assume a heterogeneous mixture of cells is stained with fluorescently labeled antibodies specific for two proteins, then analyzed on a cytometer with results shown below. A cell that expresses only one protein detected with an antibody (labeled with green fluorescence) will shift

Autoimmune Lymphoproliferative Syndrome

U : Diagnostic screen for autoimmune lymphoproliferative syndrome.

S ج ـ : 4 – 10 mL peripheral blood in sodium heparin (green top).

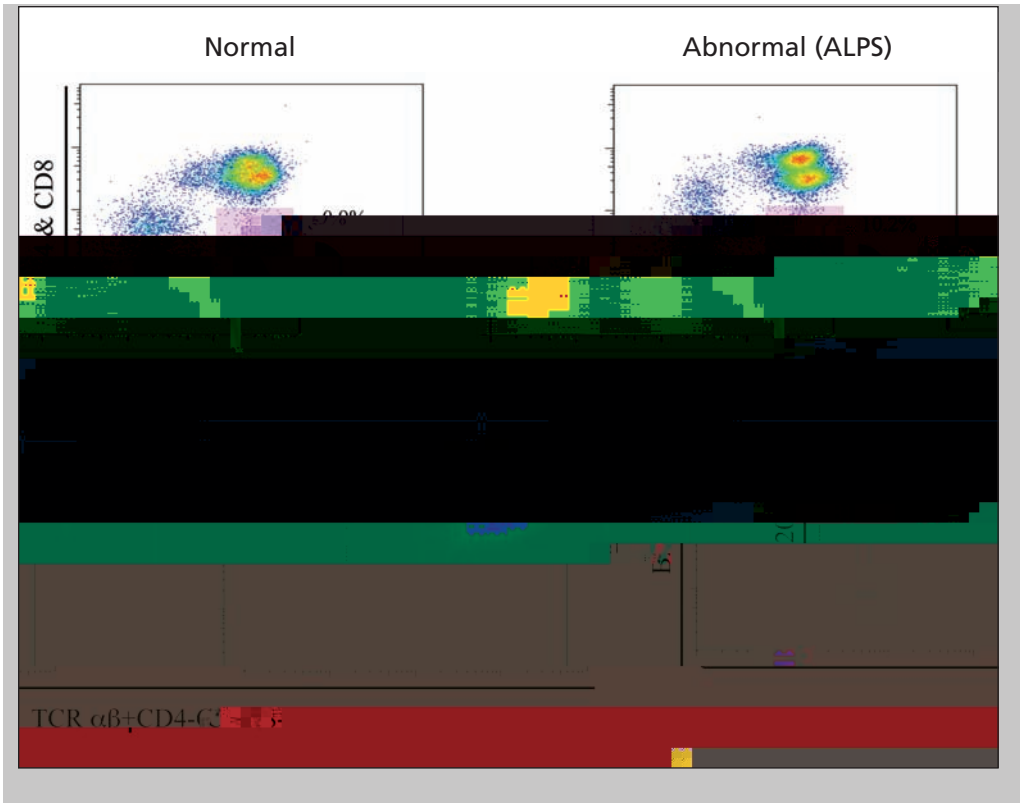
CPT c d ـ : 86359, 86360, 86355, 86357, 88184, 88185 x 11, 88188.

Clinical indication/general description

Autoimmune lymphoproliferative syndrome (ALPS), also known as Canale-Smith syndrome, is caused by a defect in apoptosis (programmed cell death) of lymphocytes via the Fas pathway leading to the abnormal accumulation of lymphocytes. Patients with ALPS present clinically with lymphadenopathy, hepatosplenomegaly and autoimmunity (autoimmune cytopenias and other autoimmune disorders) and have an increased, long-term risk to develop lymphomas.

Detection methodology

Normally, less than 1 percent of T cells that express the T cell receptor alpha and beta chains (TCRDzdz+) do not express either the CD4 or the CD8 co-receptors. These T cells are termed double negative T-cells (DNT). In ALPS, the number of TCRDzdz+DNT cells is increased. Additionally, the TCRDzdz+DNT in ALPS express an isoform of CD45 that usually is expressed only on B cells, the B220 antigen. An increased number of B220+, TCRDzdz+DNT cells are found in all characterized forms of ALPS.



Absolute T4

U : 1) Monitor treatment efficacy for human immunodeficiency virus (HIV).
2) Establish decision points for antiviral therapeutic initiation.

S ɛ ɛ : 1 – 4 mL peripheral blood in sodium heparin (green top).

CPT c d ɛ : 86359, 86360, 88184, 88185, 88187.

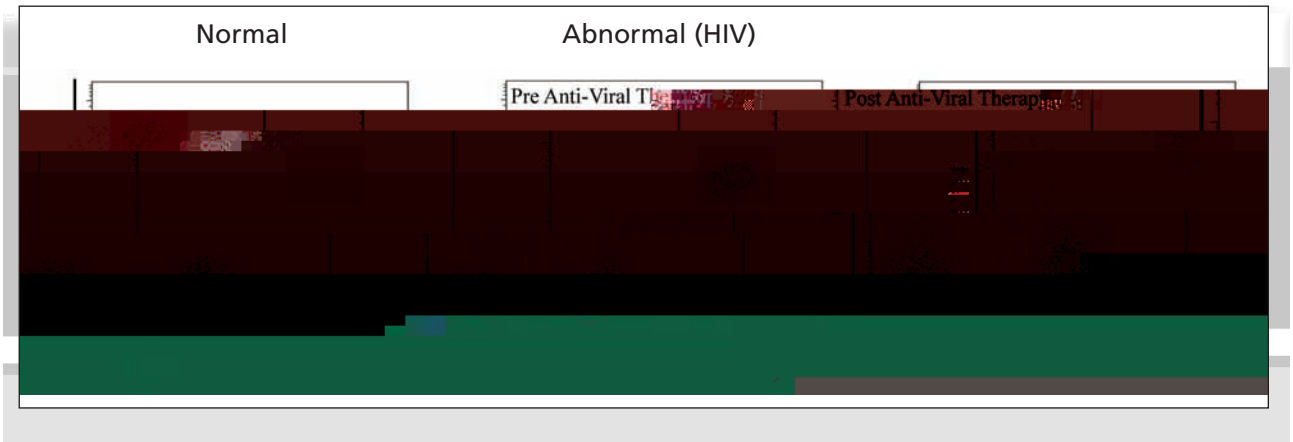
Clinical indication/general description

Human immunodeficiency virus type 1 (HIV-1) infects CD4+ T cells (helper T cells) leading to their premature death. The subsequent decrease in the number of CD4+ T cells results in the Acquired Immunodeficiency Syndrome (AIDS) and an increased susceptibility to opportunistic infections. Monitoring the number of CD4+ T cells is useful to
assess the risk of infection and to monitor the response to anti-retroviral therapy.

Detection methodology

Flow cytometric detection of CD4 and CD8 cells is used to determine the absolute CD4 and CD8 counts. AIDS presents with a severe reduction or absence of T helper cells (CD3+CD4+) with an absolute CD4 value < 200cells/ml, a low CD4:CD8 ratio (typically <1.00) and infections.

Note: The CD3 antigen exclusively is expressed on T cells.



Bruton's Tyrosine Kinase

U : 1) Diagnostic screen for X-linked agammaglobulinemia (XLA). 2) Detection of carrier status in female relative of XLA. 3) Evaluation of hypogammaglobulinemia in male patients.

S : 4 – 10 mL peripheral blood in sodium heparin (green top).

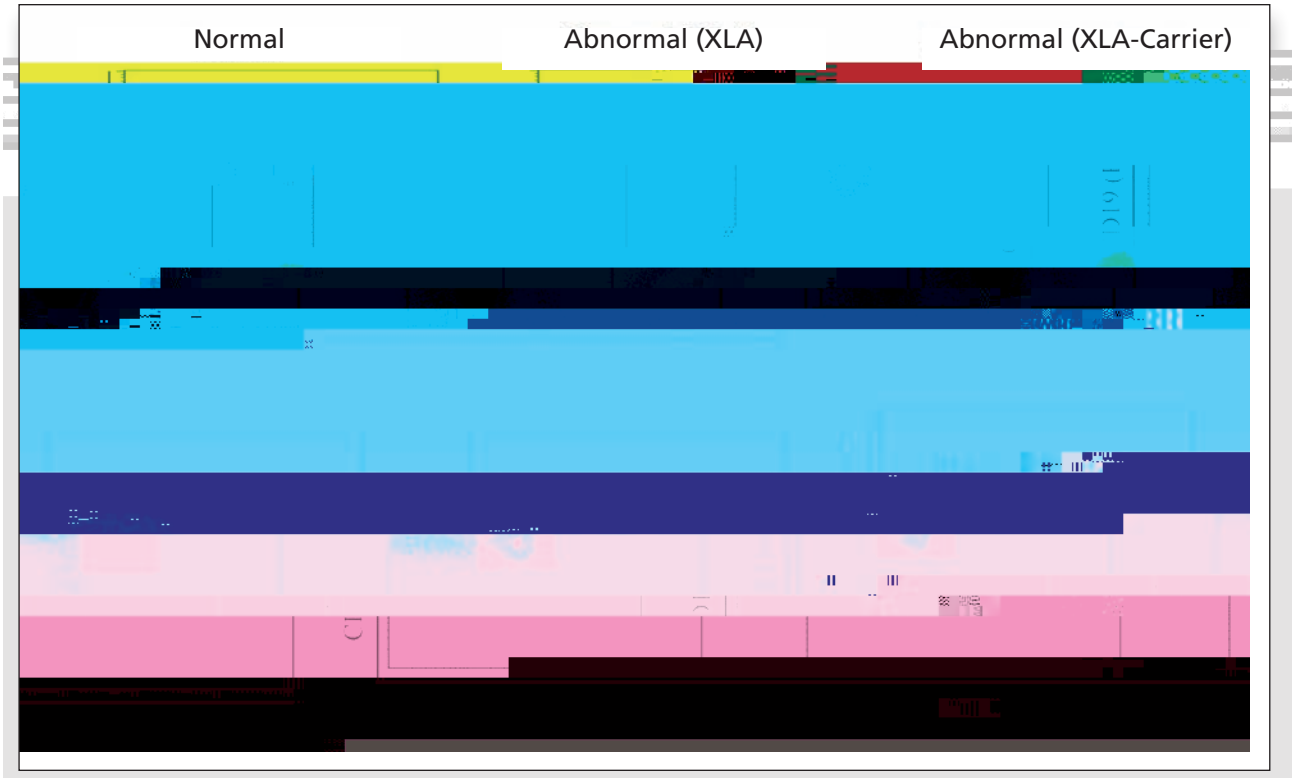
CPT c d : 86359, 86360, 86355, 86357, 88184, 88185 x 5, 88188.

Clinical indication/general description

X-linked agammaglobulinemia (XLA), also known as Bruton's agammaglobulinemia, is characterized by a marked reduction or absence of peripheral blood B cells and profound hypogammaglobulinemia of all isotypes (IgG, IgA, IgM and IgE). Patients with XLA present in early childhood with recurrent infections, in particular with encapsulated bacteria, as well as chronic enteroviral infections. XLA is caused by mutations in the Bruton's Tyrosine Kinase (BTK) gene, which is essential for the development of B cells. Some mutations in BTK result in a milder clinical and laboratory phenotype and are therefore described as leaky.

Detection methodology

XLA presents with severe reduction or absence of B cells (CD19+). Therefore, BTK protein expression is determined in CD14+ monocytes since these cells also express BTK. In XLA, monocytes express either no or very low amounts of BTK protein. Women who carry the mutated allele express normal numbers of B cells that produce normal levels of BTK due to nonrandom X inactivation. However, only 50 percent of monocytes express the BTK protein and this observation can be used to determine carrier status of relatives of affected children.



Common Variable Immunodeficiency

U : 1) Diagnostic screen for common variable immunodeficiency (CVID). 2) Assess B cell response to immunotherapeutics. 3) Assess B cell subset reconstitution post-stem cell or bone marrow transplant.

S ج ـ : 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT c d ـ : 86359, 86360, 86355, 86357, 88184, 88185 x 10, 88188.

Clinical indication/general description

CVID is characterized by a low serum IgG and either a low IgA, a low IgM or both a low IgA and IgM along with

Cytotoxicity/Apoptosis

U : 1) Diagnostic screen for hemophagocytic lymphohistiocytosis. 2) Functional evaluation of natural killer (NK) function.

S ɛ ɛ : 8 – 15 mL peripheral blood in sodium heparin (green top).

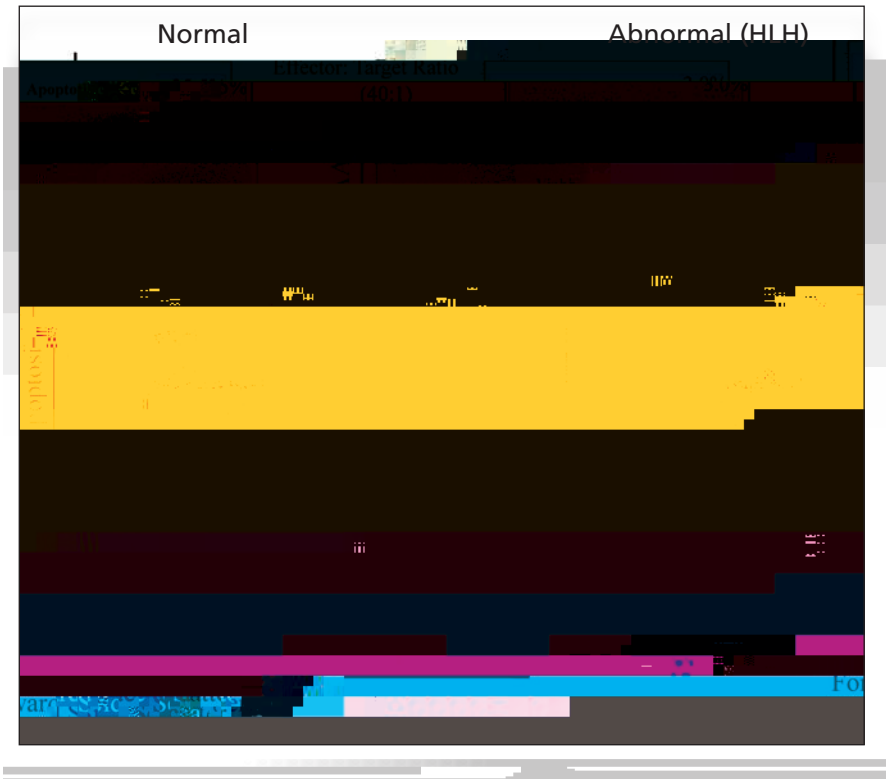
CPT c d ɛ : 86849, 88187.

Clinical indication/general description

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening disorder characterized by excessive lymphocytic activation and cytokine secretion, macrophage activation, subsequent hemophagocytosis of blood cells and organ dysfunction. This disorder usually is triggered by viral infections and typically presents at a young age. All known genetic mutations associated with HLH occur in genes encoding proteins required to kill virally infected cells. Perforin, a pore-forming protein stored in cytotoxic granules and secreted by NK cells and cytotoxic CD8+ lymphocytes, is required to kill virally infected and malignant cells. Mutations in perforin or other granule associated proteins cause HLH. Individuals with HLH exhibit defective NK cell function. In addition, defective NK cell function may be seen in patients with recurrent, severe viral infections, particularly infections with herpes viruses.

Detection methodology

A functional flow cytometric-based assay is used to evaluate the ability of a patient's NK cells to induce apoptosis of a target cell population. Target cells (K562) are fluorescently labeled to differentiate them from the patient's peripheral blood mononuclear cells. PBMCs containing NK cells (such as effectors) are cultured with target cells at different effector to target cell ratios. Target cell apoptosis is measured by incorporation of the fluorescent dye 7-AAD, which binds to the DNA in apoptotic cells. The cytotoxicity/apotosis assay will detect defects in NK cell function in patients with clinical symptoms of HLH, and it also can be used to test the function of NK cells in patients with severe or chronic viral infections.



Neutrophil Oxidative Burst

U : 1) Functional evaluation of neutrophil oxidative burst potential. 2) Diagnostic screen for chronic granulomatous disease (CGD). 3) Detection of carrier status in female relative of CGD patient.

S ɛ ɛ : 1 – 4 mL peripheral blood in sodium heparin (green top).

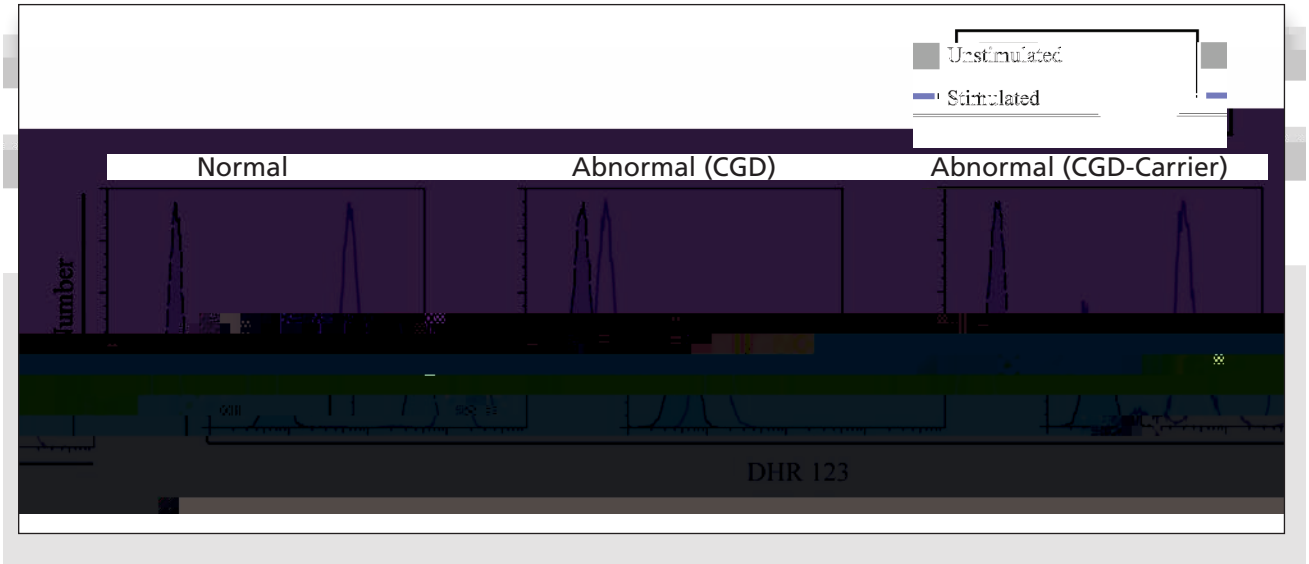
CPT c d ɛ : 88184, 88185, 87187.

Clinical indication/general description

CGD is a group of disorders characterized by a defective oxidative burst resulting in an inability to generate toxic oxygen radicals (superoxide) that are required to kill bacteria. Patients affected by this disorder present with recurrent bacterial infections or abscesses, particularly of the skin, subcutaneous areas or regional lymph nodes. In CGD, microbial killing is defective due to mutations in one of four known components of the NADPH oxidase system: one X-linked (gp91-phox) and three autosomal recessive (p22-phox, p47-phox and p67-phox).

Detection methodology

This is a flow cytometric functional assay used to assess the ability of neutrophils to produce an oxidative burst. Neutrophils are loaded with dihydrorhodamine (DHR) dye and then activated with phorbol-12-myristate-13 acetate (PMA). Normal activated neutrophils produce superoxides that oxidize DHR resulting in the emission of fluorescence that is quantitated by flow cytometry. Neutrophils from patients with CGD cannot generate superoxide and therefore do not oxidize DHR.

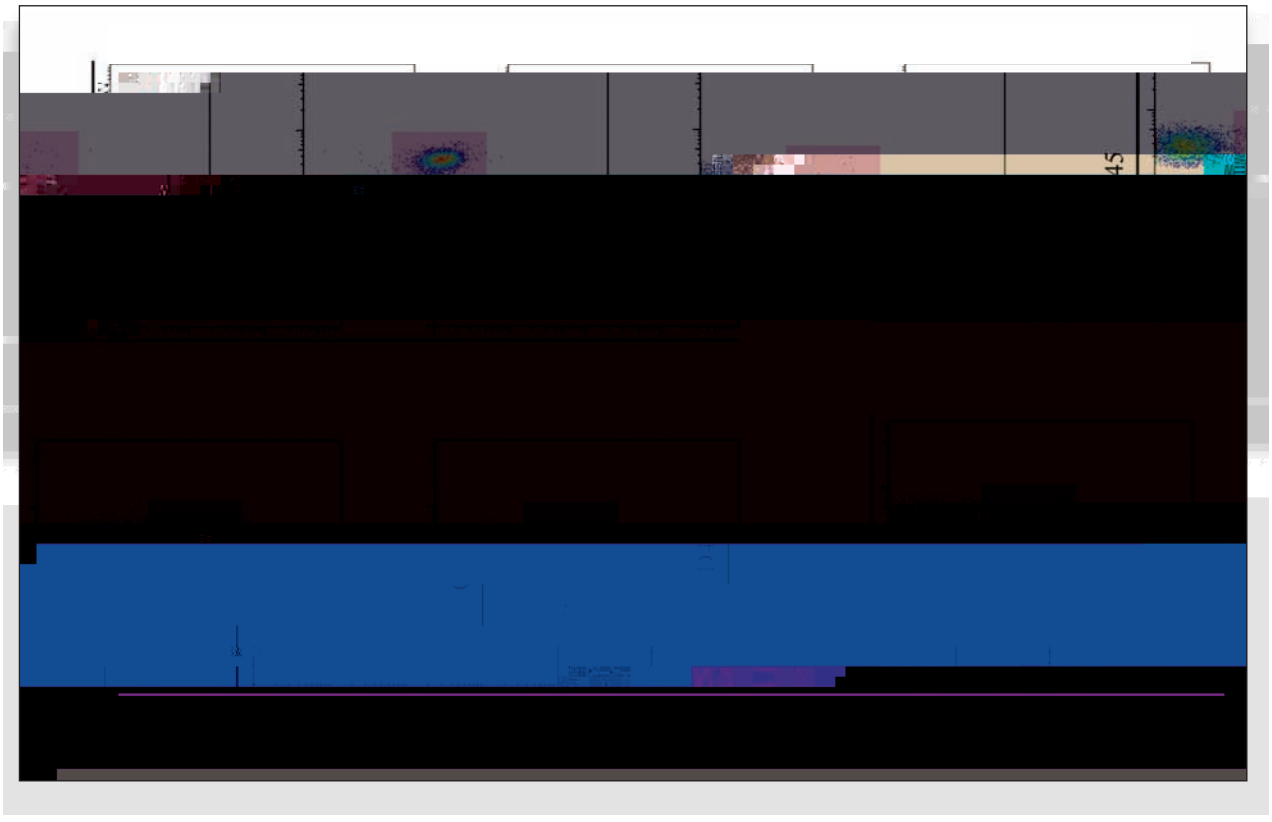


Neutrophil Phagocytosis

U : Functional evaluation of neutrophil phagocytosis.

S ع ج : 1 – 4 mL peripheral blood in sodium heparin (green top).

C ج :



Primary Immunodeficiency 1

U : General evaluation for T, B and natural killer (NK) cell populations.

S ج ـ : 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT c d ـ : 86359, 86360, 86355, 86357, 88184, 88185 x 4, 88187.

Clinical indication/general description

Primary immunodeficiencies (PIDDs) and secondary immunodeficiencies present with recurrent upper and lower respiratory tract infections (encapsulated and atypical bacteria), deep-seated infections, recurrent or deep-seated abscesses, intractable diarrhea and failure to thrive. In addition, PIDDs also can present with autoimmune manifestations and malignancies. Evaluation of patients with these manifestations includes enumeration of the different types of lymphocytes (T cells, B cells and NK cells) because an absolute lymphocyte count from a complete blood count differential can miss important deficiencies in specific subsets of lymphocytes.

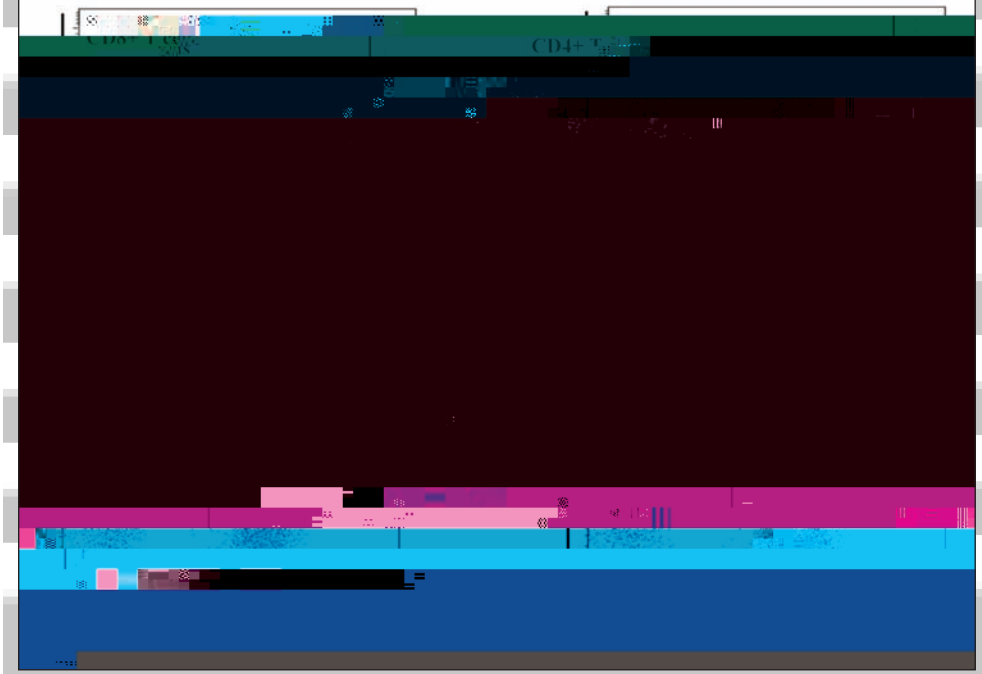
The PID1 assay enumerates the numbers of helper (CD4) and cytotoxic (CD8) T cells, B cells and NK cells. Numerous immunodeficiencies associated with decreased numbers of T cells, B cells or NK cells can be detected with this assay including DiGeorge syndrome (low T cell numbers will be detected), AIDS (low CD4 cell counts will be detected), X-linked agammaglobulinemia (low B cells will be detected) and NK cell deficiencies (low NK cell numbers will be detected).

Detection methodology

The assay is designed to enumerate the percent and absolute cell counts of T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+/CD56+).

Note: This assay does not detect neutrophil adhesion defects (leukocyte adhesion deficiency) or signs of immune activation.

Typical Normal Expression Patterns



Severe Combined Immunodeficiency

U : Confirmatory test of severe combined immunodeficiency (SCID) newborn screening.

S ج ـ : 1 – 2 mL peripheral blood in sodium heparin (green top).

CPT c d ـ : 86359, 86360, 86355, 86357, 88184, 88185, 81887.

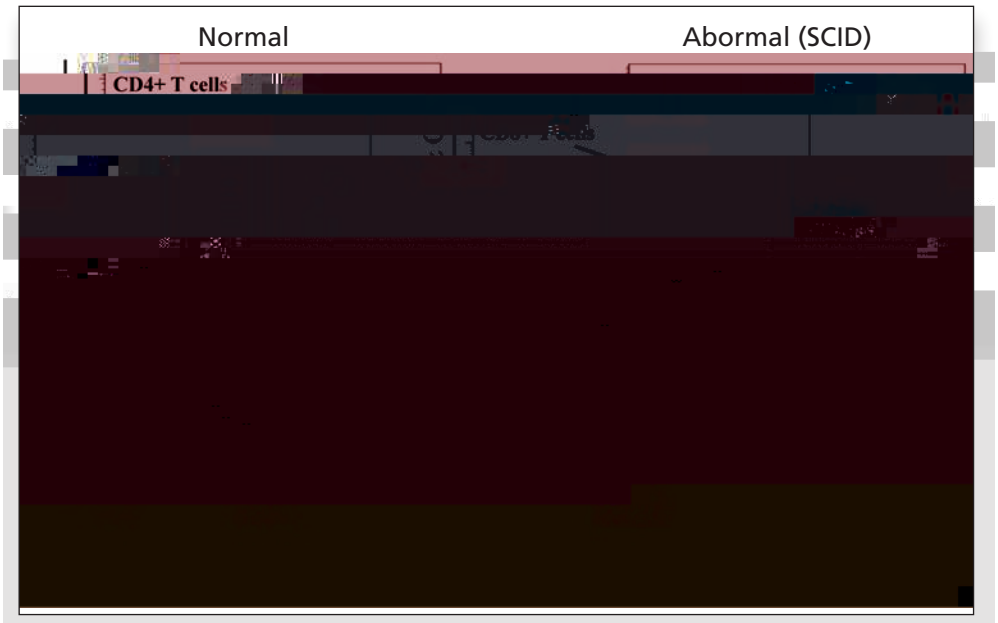
Clinical indication/general description

SCID is characterized by profound impairment of both cellular and humoral immunity due to the absence or markedly diminished number of T cells and variably decreased numbers of B cells or natural killer (NK) cells. Infants affected with SCID typically present within the first year of life with recurrent bacterial and viral infections, opportunistic infections (such as pneumocystis pneumonia), and can have fatal responses to live viral vaccination. SCID is life threatening within the first year of life if not detected and treated with hematopoietic stem cell transplantation. This is a limited assay that only enumerates the numbers of CD4+ T cells, CD8+ T cells, B cells and NK cells. This assay is designed as a confirmatory immunophenotyping for infants who fail the newborn screening program for SCID in the state of Wisconsin.

Detection methodology

The assay is designed to enumerate the percent and absolute cell counts of T helper cells (CD3+CD4+), T cytotoxic cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+/CD56+). This assay also enumerates the percentage of naïve CD4+ and CD8+ T cells.

Note: The assay should only be ordered for confirmatory immunophenotyping as part of the state of Wisconsin's newborn screening program for SCID.



T cell Activation

U : Functional evaluation of T cell activation.

S ج ـ : 8 – 15 mL peripheral blood in sodium heparin (green top).

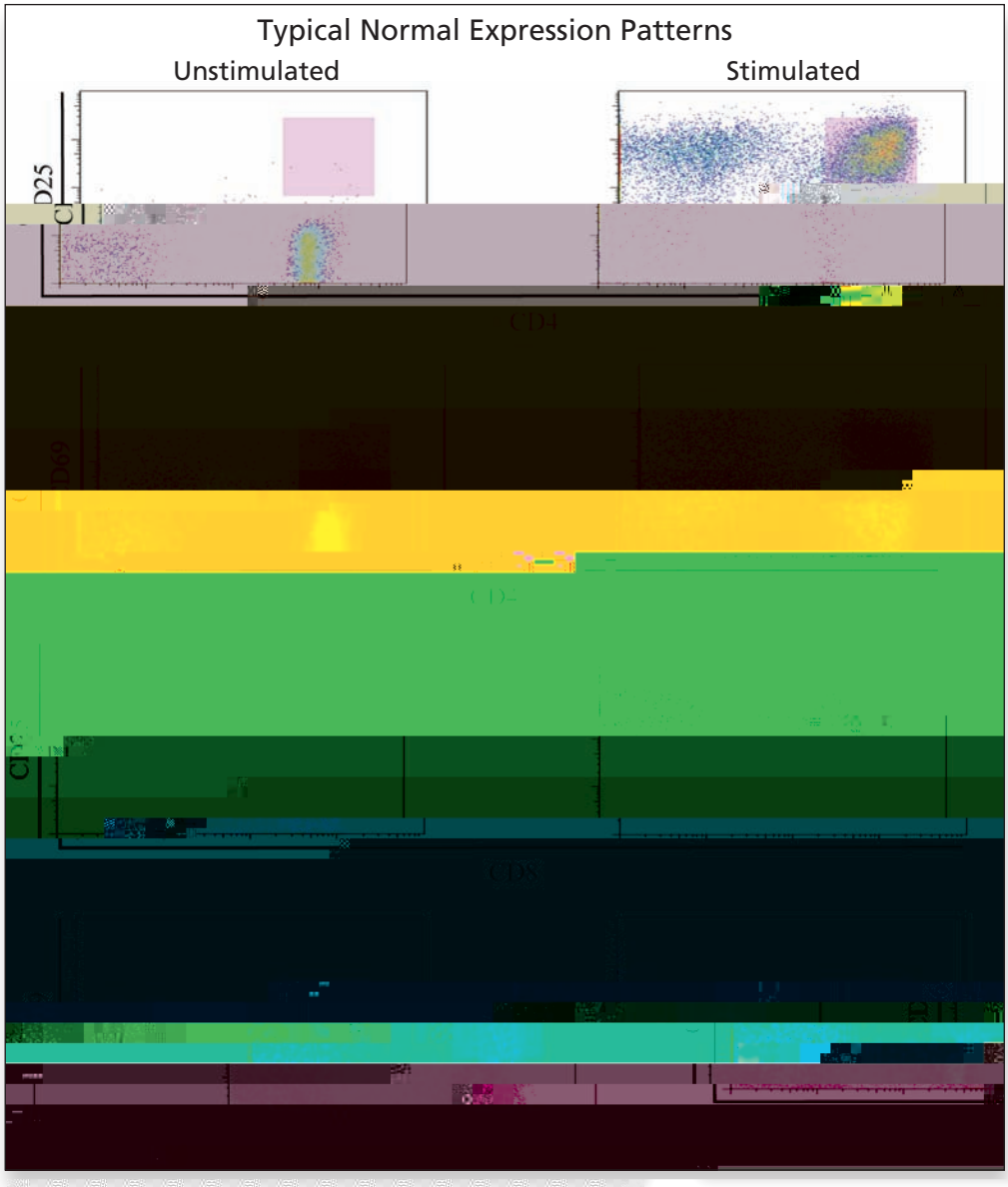
CPT c d ـ : 86359, 86360, 86355, 86357, 88184, 88185 x 6, 81887.

Clinical indication/general description

When T cells are activated through the T cell receptor, a number of signal transduction events occur leading to the expression of the surface marker CD69 at early time points (4 hours) and CD25 at late time points (4 days). This assay can be used to evaluate infants with defective T cell function.

Detection methodology

A functional flow cytometric-based assay evaluates activation of T helper cells (CD3+CD4+) and T cytotoxic cells (CD3+CD8+) using CD25 and CD69 surface expression. Quiescent lymphocytes express low levels of the CD25 and CD69 antigens and upon activation the expression of CD25 and CD69 is markedly increased.



T helper IL17

U : Diagnostic screen for hyper – immunoglobulin E syndrome (HIES).

S ج : 4 – 10 mL peripheral blood in sodium heparin (green top).

CPT c d ج: 86359, 86360, 86355, 86357, 88184, 88185 x 6, 81887.

Clinical indication/general description

HIES, also known as Job's syndrome, is characterized by pulmonary infections, staphylococcal abscesses, eczema and abnormalities of bone and connective tissue. IgE levels typically are very high. HIES syndrome can look very similar to severe eczema, thus a laboratory test to differentiate these syndromes is clinically useful. The defects in Hyper-IgE syndrome are caused by mutations in the transcription factor *STAT3*. STAT3 is required to induce CD4+ T cells to produce IL-17, a cytokine that is important for the elicitation of an effective immune response to several bacteria and fungi. The T helper IL-17 funtional assay measures the ability of CD4+ T cells to make IL-17, which is defective in patients with HIES.

Detection methodology

Peripheral blood mononuclear cells (PBMCs) are activated *in vitro* with PMA and ionomycin to induce the expression of IL-17 in normal T helper cells (CD3+CD4+), which is measured by flow cytometry using antibody that specifically recognizes IL-17. Simultaneously, IFN- is measured within the T cytotoxic cell (CD3+CD8+) as a control to ensure adequate activation of T cells. An extremely low percentage of IL-17+ CD4+ T cells is associated with HIES, whereas the percentage of IL-17+ CD4+ cells is normal or slightly reduced in eczema.

Toll-like Receptor

U : 1) Functional evaluation of toll–like receptors (TLRs). 2) Diagnostic screen for primary

T cell Mitogen Proliferation

U : Functional evaluation of T cells proliferation to mitogens.

S ج ـ : 8 – 15 mL peripheral blood in sodium heparin (green top).

CPT c d ـ : 86353 x 5, 88187.

Clinical indication/general description

Once a defect in T cells is detected, or defective T cell function is suspected, further evaluation involves examination of T cell proliferation in response to mitogens. Activation of T cells with antibodies to the T cell receptor or with plant lectins results in the proliferation of these cells over the next seven to 10 days. Diminished or absent proliferative response to T cell stimuli is consistent with a primary or secondary immunodeficiency disease that affects T lymphocytes (cellular immunity). This assay tests the proliferative function of T cells to T cell receptor antibodies and a variety of mitogens.

Detection methodology

A functional flow cytometric-based assay provides a semi-quantitative assessment of lymphocyte proliferation in response to concanavalin A (ConA)/IL-2, phytohemagglutinin (PHA)/IL-2, phorbol esters and soluble CD3. Lymphocytes are labeled with the fluorescent dye CFSE and activated with mitogens. As lymphocytes divide, the fluorescent label is diluted in half, which can be seen on flow diagrams as peaks of decreasing fluorescence. Lymphocyte proliferative response is demonstrated by an increase in FSC/SSC and a progressive two-fold reduction in a proliferation tracking dye. Lymphocyte proliferative response is demonstrated by an increase in FSC/SSC and a progressive two-fold reduction in a proliferation tracking dye.

T Regulatory – FOXP3 (TREG)

U : 1)

X-linked Lymphoproliferative Syndrome (XLP)

U : 1) Evaluate the presence of SLAM-associated protein (SAP) and X-linked inhibitor of apoptosis (XIAP) in peripheral blood. 2) Diagnostic screen for X-Linked Lymphoproliferative Syndrome (XLP), types 1 and 2.

Test name	Code	Diagnostic utility
Autoimmune Lymphoproliferative Syndrome	AILYMP	Screen for ALPS.
Absolute T4	AT4	Monitor treatment efficacy for HIV.
Bruton's Tyrosine Kinase	BTK	• Screen for X-linked agammaglobulinemia. • Carrier status detection in female relative of XLA.
Common Variable Immunodeficiency	CVID	Screen for CVID.
Cytotoxicity/Apoptosis	CYTAPO	• Screen for hemophagocytic lymphohistiocytosis. • Functional evaluation of natural killer function.
Hyper IGM	HIGM	• Screen for X-linked (CD40L) Hyper IgM. • Screen for autosomal recessive (CD40) Hyper IgM. • Carrier status detection in female relative of XL-HIGM.
Neutrophil Oxidative Burst	NEUOXB	• Functional evaluation of neutrophil oxidative burst. • Screen for chronic granulomatous disease. • Carrier status detection in female relative of CGD.
Neutrophil Phagocytosis	PHAGO	Functional evaluation of Neutrophil Phagocytosis.
Perforin-Granzyme	PERGRA	• Screen for hemophagocytic lymphohistiocytosis. • Perforin, granzyme A, granzyme B detection in lymphocytes.

Test name	Code	Diagnostic utility
Primary Immunodeficiency 1	PID 1	

Primary immunodeficiency diagnosis and treatment

While identification of primary immunodeficiency diseases can be difficult, timely diagnosis and treatment prevents complications and saves lives. We can help.

Clinical Immunodiagnostic and Research Laboratory

The Medical College of Wisconsin

Our lab:

- Offers a comprehensive array of tests to aid in diagnosis of PID and other hematopoietic disorders.
-