Jeffrey Modell Foundation Mission

Vicki and Fred Modell established the Je□rey Modell Foundation, a 501(c)(3) nonprofit organization, in 1987, in memory of their son Je□rey, who died at the age of 15 from complications of primary immunodeficiency − a genetic condition that is chronic, serious and often fatal. JMF is a global patient organization devoted to early and precise diagnosis, meaningful treatments and ultimately, cures through clinical and basic research, physician education, patient support, advocacy and public awareness. The Foundation has developed a global network of more than 100 Je□rey Modell Diagnostic and Research Centers worldwide − consisting of 459 expert physicians at 195 academic institutions in 58 countries and spanning 6 continents − and continues to expand globally.

The Foundation's website, info4pi.org, o□ers resources to patients, families and health care providers.

Table of contents

Introduction

The purpose of this booklet is to inform physicians of the commonly used flow cytometric tests used for the evaluation of primary immune deficiency 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 human immunology has progressed.

The evaluation of primary immune deficiency disorders has benefited greatly 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 di□erent components of the immune system are possible. This educational pamphlet will give 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 where 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. Di□erent fluorescent compounds emit light at di□erent wavelengths, which allows for the discrimination of several di□erent proteins on a cell. When multiple fluorescent compounds are used to analyze a cell population, the results are typically depicted in two-dimensional diagrams (two-parameter dot plot or one-parameter histogram). 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 along the X-axis but not the Y-axis. A cell that expresses only one protein detected with an antibody (labeled with red fluorescence) will shift along the Y-axis but not the X-axis. A cell expressing both antigens will shift along both X- and Y-axes. These cells are referred to as “double positive” cells. Cells that do not express either antigen will not shift along either X- or

Autoimmune Lymphoproliferative Syndrome (AILYMP)

Utility

- Diagnostic screen for autoimmune lymphoproliferative syndrome

Clinical indication/general description

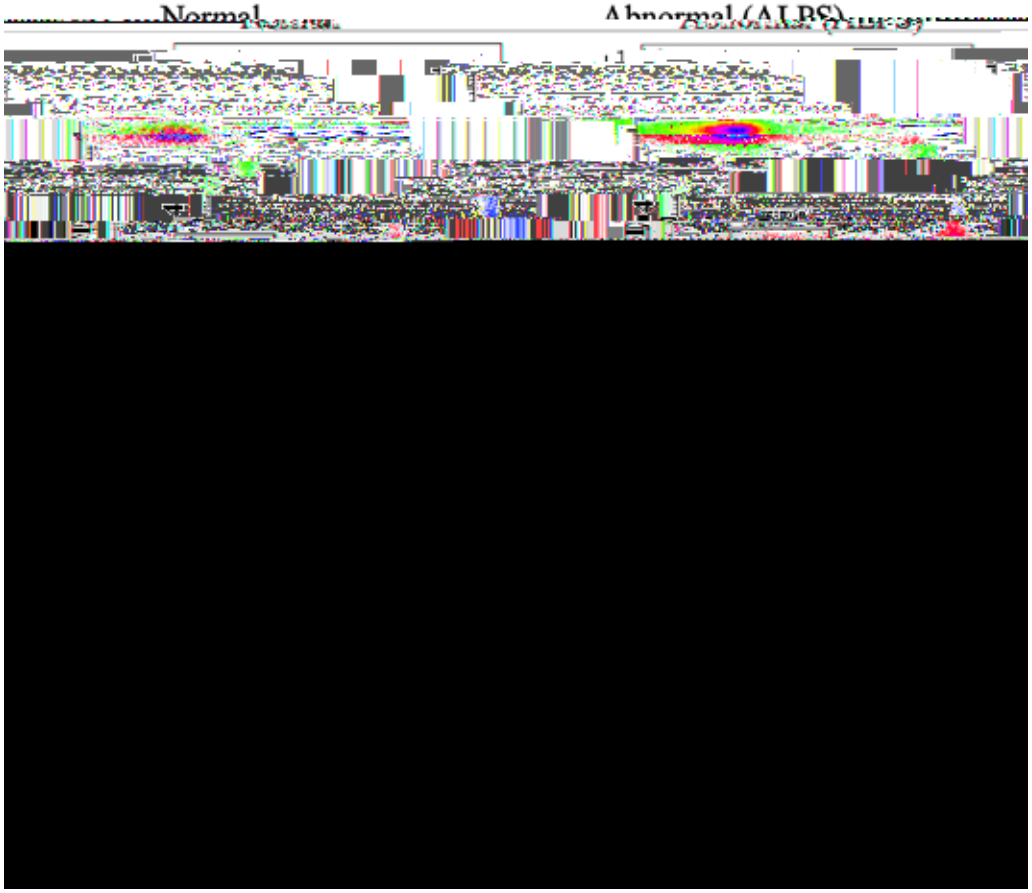
Autoimmune lymphoproliferative syndrome (ALPS, also known as Canale-Smith syndrome) is caused by a defect in apoptosis (programmed cell death) of lymphocytes via the FAS pathway, leading to the abnormal accumulation of lymphocytes. Patients with ALPS present clinically with lymphadenopathy, hepatosplenomegaly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

Detection methodology

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

Markers

CD3/CD4/CD8/B220/CD19/CD21/CD27/HLA-DR/IgD/CD16/CD56/CD14/CD45/ IgM/ TCR / TCR / CD45RA/CD45RO



Bruton's Tyrosine Kinase (BTK)

Utility

- Diagnostic screen for X-linked agammaglobulinemia (XLA)
- Detection of carrier status in female relative of XLA
- Evaluation of hypogammaglobulinemia in male patients

Clinical indication/general description

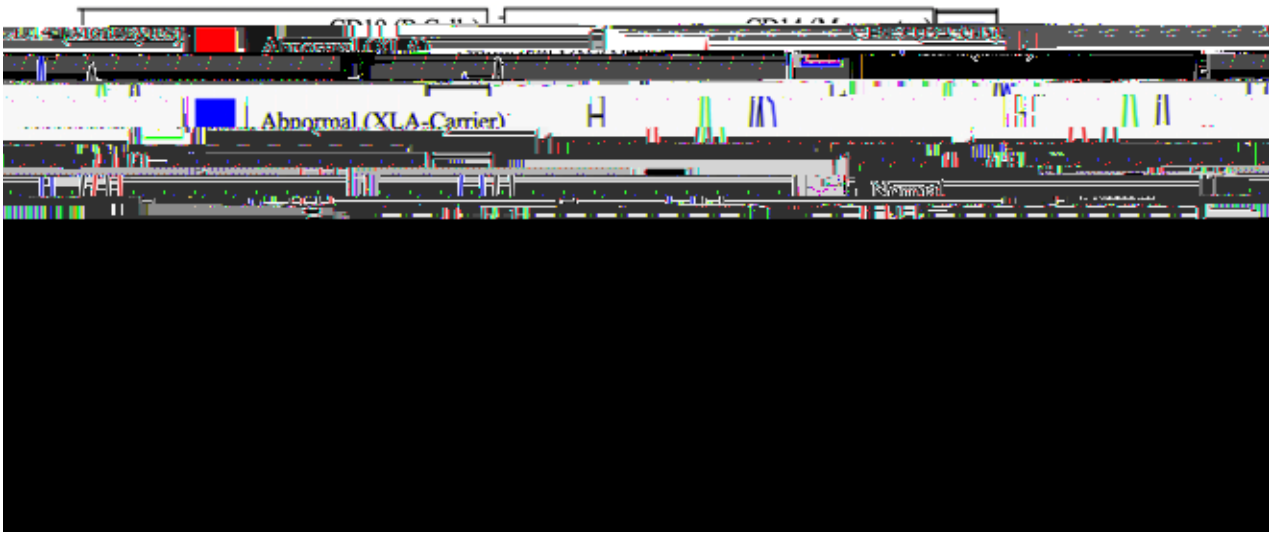
X-linked agammaglobulinemia (XLA), also known as Bruton's agammaglobulinemia, is characterized by a marked reduction or absence of peripheral blood B cells and profound hypogammaglobulinemia of all isotypes (IgG, IgA, IgM, and IgE). Patients with XLA present in early childhood with recurrent infections, especially with encapsulated bacteria, as well as chronic enteroviral infections and enteric bacterial 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

X-linked agammaglobulinemia (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 express normal levels of BTK due to non-random X inactivation. However, only 50% of monocytes express the BTK protein, and this observation can be used to determine carrier status of relatives of a□ected children.

Markers

CD3/CD4/CD8/CD19/BTK/CD16/CD56/CD14/CD45



Common variable immunodeficiency (CVID)

Utility

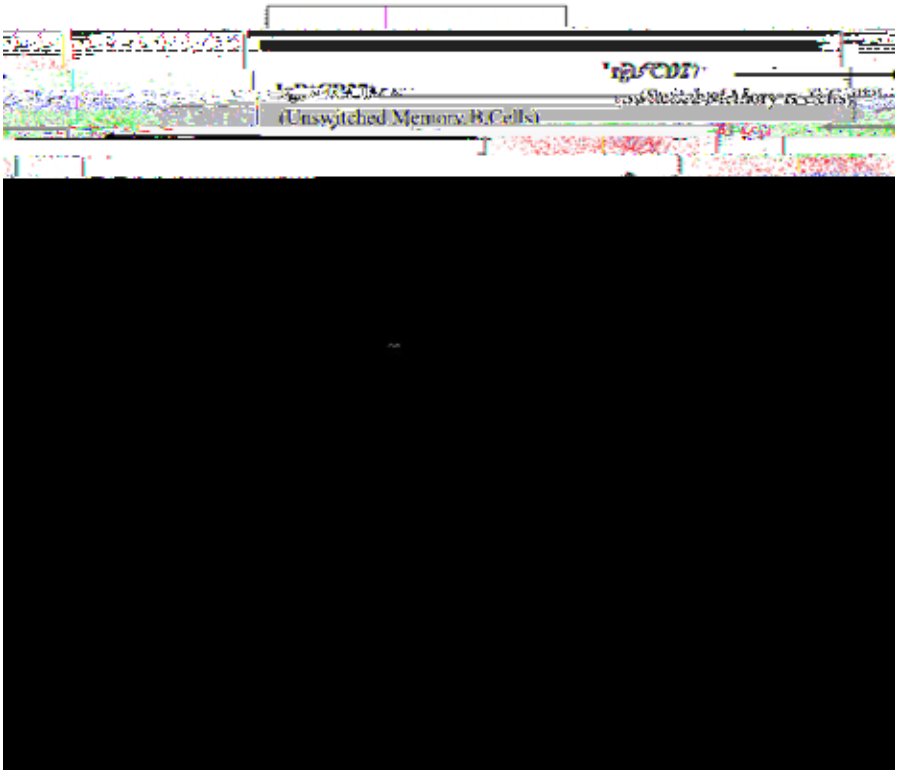
- Diagnostic screen for common variable immunodeficiency (CVID)
- Assess B cell response to immune therapeutics
- Assess B cell subset reconstitution post-stem cell or bone marrow transplant

Clinical indication/general description

Common variable immunodeficiency (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 an impaired ability to make specific antibodies in response to immunization. CVID may occur at any age, and such patients have recurrent respiratory tract infections with encapsulated bacteria and Mycoplasma sp. B cell numbers may be normal or decreased, and T cell numbers may also be reduced. Patients with CVID are at increased risk to develop granulomatous or lymphocytic interstitial lung disease (GLILD), autoimmunity (autoimmune thrombocytopenic purpura, autoimmune hemolytic anemia) and lymphomas.

Detection methodology

The CVID assay analyzes B cell maturation, which occurs in a sequential immunophenotypic pattern (illustrated below). B cells that are naïve (antigen inexperienced) do not express CD27. Upon engagement with antigen, B cells express CD27 and are termed memory B cells. Memory B cells are further classified as unswitched memory B cells (express CD27 and IgD) and switched memory B cells (express CD27 but do not express IgD). Patients with CVID that have a markedly reduced number of switched memory B cells are at increased risk to have a more severe clinical phenotype and to develop GLILD. Low numbers of switched memory B cells can also be seen in other disorders a□ecting B cell maturation, such as hyper-IgM syndrome. This assay also defines B cell subsets as detailed by the EUROclass criteria, including CD38, IgM, etc.



Markers

CD3/CD4/CD8/CD45RA/ CD45RO/CD21/CD27/HLA-DR/IgD/CD16/CD56/CD14/ CD45/CD38/IgM/CD24

Cytokine IBD (CYTIBD)

Utility

- Diagnostic screen for inflammatory bowel disease (IBD)
- Evaluates IL-10 receptor function
- Evaluates STAT3 function (Hyper IgE)
- Determines the ability of anti-inflammatory cytokine IL-10 to suppress endotoxin-induced cytokine storm

Clinical indication/general description

The inability of IL-10 to mitigate the synthesis and secretion of the pro-inflammatory cytokine

Cytotoxicity/Apoptosis (CYTAPO)

Utility

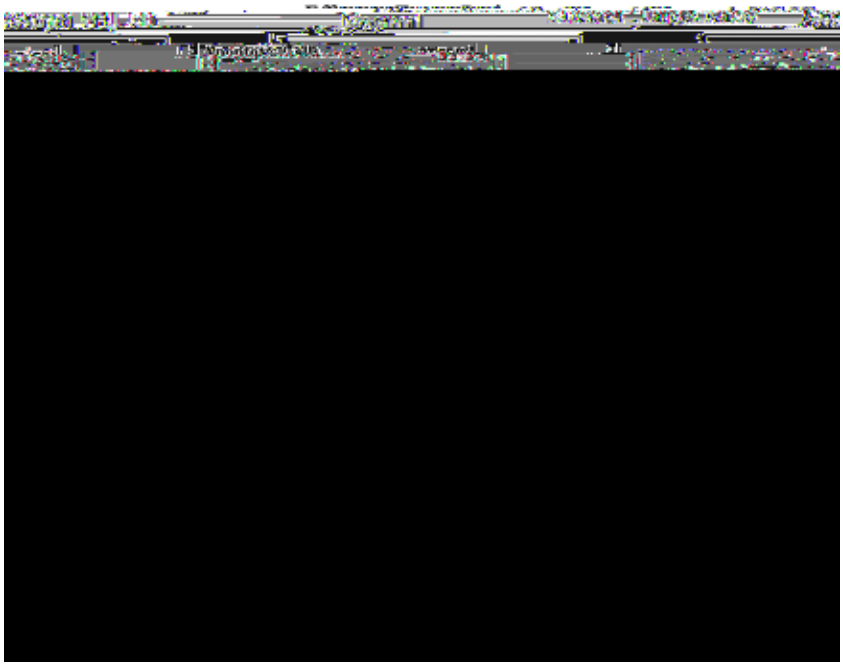
- Diagnostic screen for hemophagocytic lymphohistiocytosis
- Functional evaluation of natural killer (NK) function

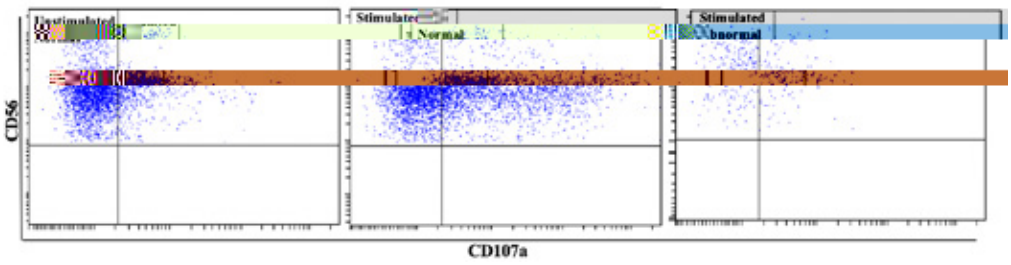
Clinical indication/general description

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 is usually 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 natural killer (NK) cell function. In addition, defective NK cell function may be seen in patients with recurrent, severe viral infections, particularly infections with herpes viruses. In addition, CD107 (LAMP-1) is upregulated upon NK degranulation.

Detection methodology

A functional flow cytometric-based assay is used to evaluate the ability of a patient's natural killer (NK) cells to induce apoptosis of a target cell population. Target cells (K562) are fluorescently labeled to di□erentiate them from a patient's PBMCs. PBMCs containing NK cells (e□ectors) are cultured with targets at di□erent e□ector to target cell ratios, and 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 can also be used to test the function of NK cells in patients with severe or chronic viral infections.





Markers

CD8/CD56/CD16/CD107a

Functional Asplenia/Howell-Jolly Body Detection (FAHJB)

Utility

- Aid in the diagnosis of splenic dysfunction by analyzing erythrocytes to detect the presence of micronuclei (Howell-Jolly bodies)
- Patients with hyposplenism are unable to filter out micronucleated red blood cells (RBCs); thus, these cells are found in the peripheral circulation in increased numbers.

Clinical indication/general description

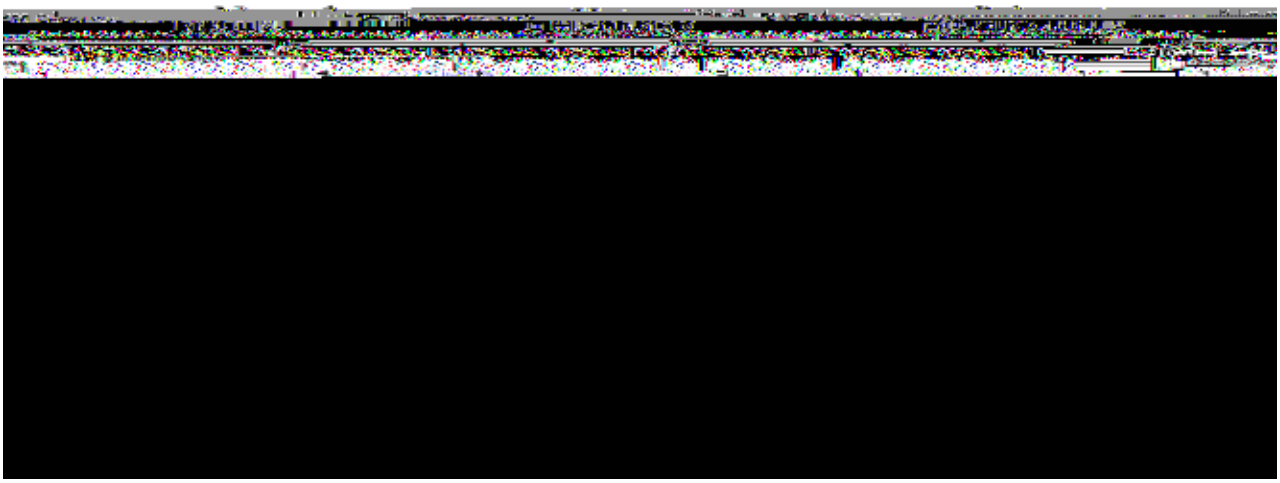
The loss of splenic function can result in life-threatening complications, such as infections. There are a number of conditions that could result in splenic dysfunction, such as heterotaxy, and it can be hard to detect splenic issues in these cases.

Detection methodology

Determination of functional asplenia is performed using the reagent propidium iodide (PI) to detect small fragments of DNA known as Howell-Jolly bodies (HJB) within mature erythrocytes. Normally, as red blood cells transition from the reticulocyte stage to a mature red blood cell, the remaining nuclear material is extruded from the cell. Patients with a spleen that is not functioning properly will exhibit an increase in circulating mature red blood cells staining positive for PI, indicating the presence of DNA within the cells. In this assay, we stain the cells with CD45 to exclude white blood cells, CD71 to stain immature red blood cells and CD61 to exclude platelets.

Markers

CD45/CD61/CD71



Mendelian Susceptibility to Mycobacterial Diseases (MSMD)

Utility

- Evaluates susceptibility to intracellular bacterial pathogens such as Mycobacterium, Leishmania, Listeria species, non-pathogenic (atypical) mycobacteria (M. tuberculosis, M. africanum, M. microti, M. bovis) and certain viruses (vaccinia virus)
- Detects IFN R1, IFN R2, IL12R 1, IL12p40, STAT1 and STAT4 defects

Clinical indication/general description

Neutrophil Phenotype and Function (NPF)*

Utility

- Functional evaluation of neutrophil oxidative burst potential
- Diagnostic screen for chronic granulomatous disease (CGD)
- Detection of carrier status in female relative of CGD patient
- Leukocyte adhesion deficiencies

Clinical indication/general description

Chronic granulomatous disease (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 a□ected 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 is X-linked (gp91-phox) and three are autosomal recessive (p22-phox, p47-phox, and p67-phox). Leukocyte adhesion deficiency Type I (LAD-I), also known as LFA-1 deficiency, is caused by a decreased expression of CD18. Leukocyte adhesion deficiency Type II (LAD-II) is caused by the decreased expression of CD15.

Detection methodology

This functional flow cytometric assay is 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

Perforin-Granzyme (PERGRA)

Utility

- Determination of intracellular perforin and granzyme B with cytolytic lymphocytes

Clinical indication/general description

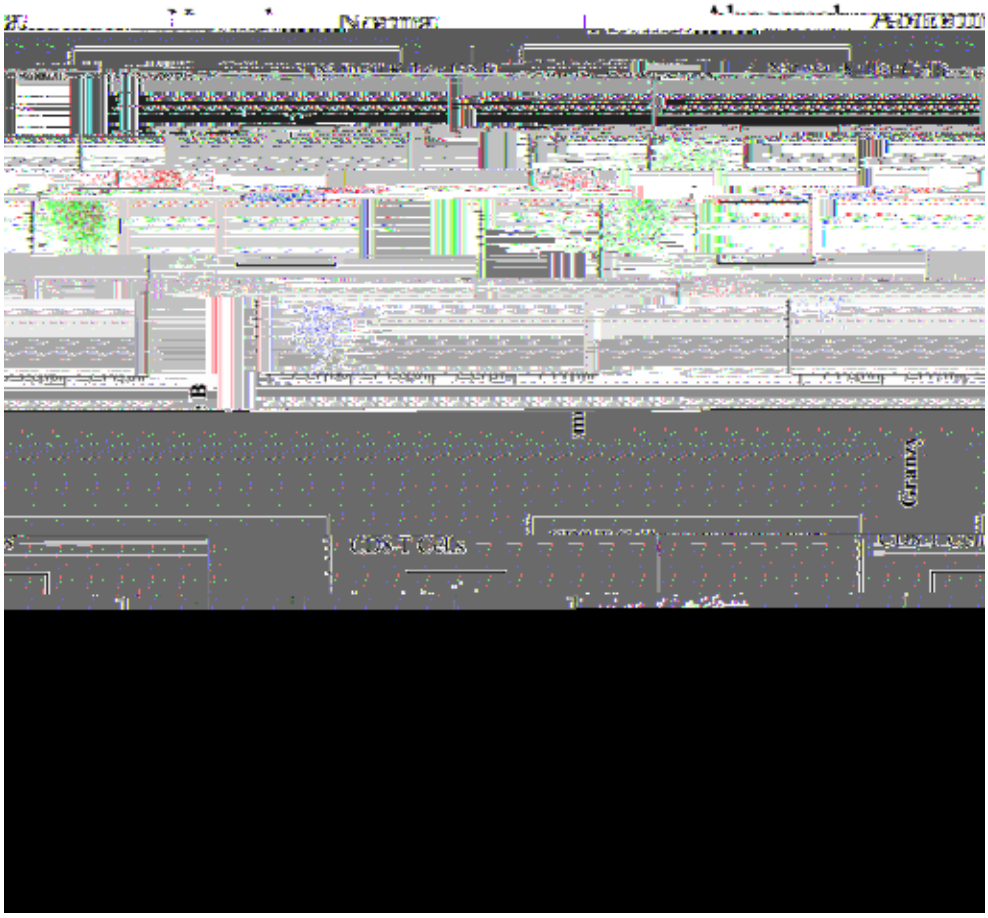
Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening disorder characterized by excessive lymphocytic activation and cytokine secretion, macrophage activation, subsequent hemophagocytosis of blood cells, and organ dysfunction. This disorder is usually triggered by viral infections and typically presents at a young age. All known genetic mutations associated with HLH occur in genes encoding proteins in NK cells and cytotoxic lymphocytes that are required to kill virally infected cells. Perforin, a pore-forming protein stored in cytotoxic granules and secreted by NK cells and cytotoxic lymphocytes, is required to kill virally infected and malignant cells. Mutations in perforin as well as other granule-associated proteins have been shown to cause HLH. Individuals with HLH exhibit defective natural killer (NK) cell function.

Detection methodology

The assay is designed to enumerate the percentage of natural killer cells (CD56+CD16-) and T cytotoxic cells (CD56-CD8+) expressing perforin and granzyme B. This assay detects patients with HLH that lack perforin and is used to evaluate the expression of cytotoxic molecules (perforin and granzyme B) in NK cells.

Markers

CD3/CD4/CD8/
CD19/CD56/CD16/
CD14/CD45/
Granzyme B/
Perforin



PHOX

Utility

- To assess expression of the NADPH oxidase complex
- Confirmation of oxidative burst defects and genetic defects (important for the p47 subunit, which is di□cult to sequence)

Clinical indication/general description

Assessment of expression of the NADPH oxidase subunits is used to confirm loss of expression when functional testing (DHR) is abnormal. This can also be used to assess any genetic defects in this pathway. Its important to note that the p47-PHOX subunit has a pseudogene, which can make sequencing di□cult.

Detection methodology

Whole blood is stained intracellularly with antibodies against the subunits and staining in neutrophils assessed.

Markers

CD14/CD45/p40/p47/p67/p22/p90

Primary Immunodeficiency 1 (PID1)

Utility

- General evaluation for T, B and natural killer (NK) cell populations

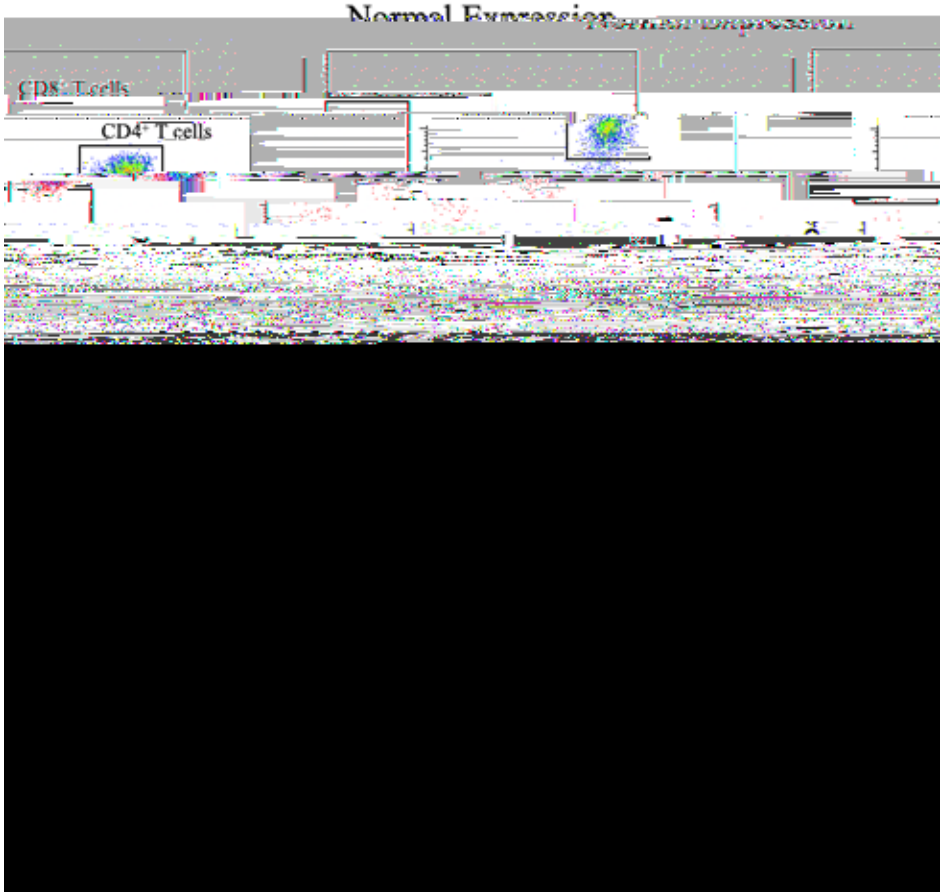
Clinical indication/general description

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 can also present with autoimmune manifestations and malignancies. Evaluation of patients with these manifestations includes enumeration of the di□erent types of lymphocytes (T cells, B cells, NK cells) because an absolute lymphocyte count from a complete blood count di□erential 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 natural killer cells (CD3-CD16+/CD56+).

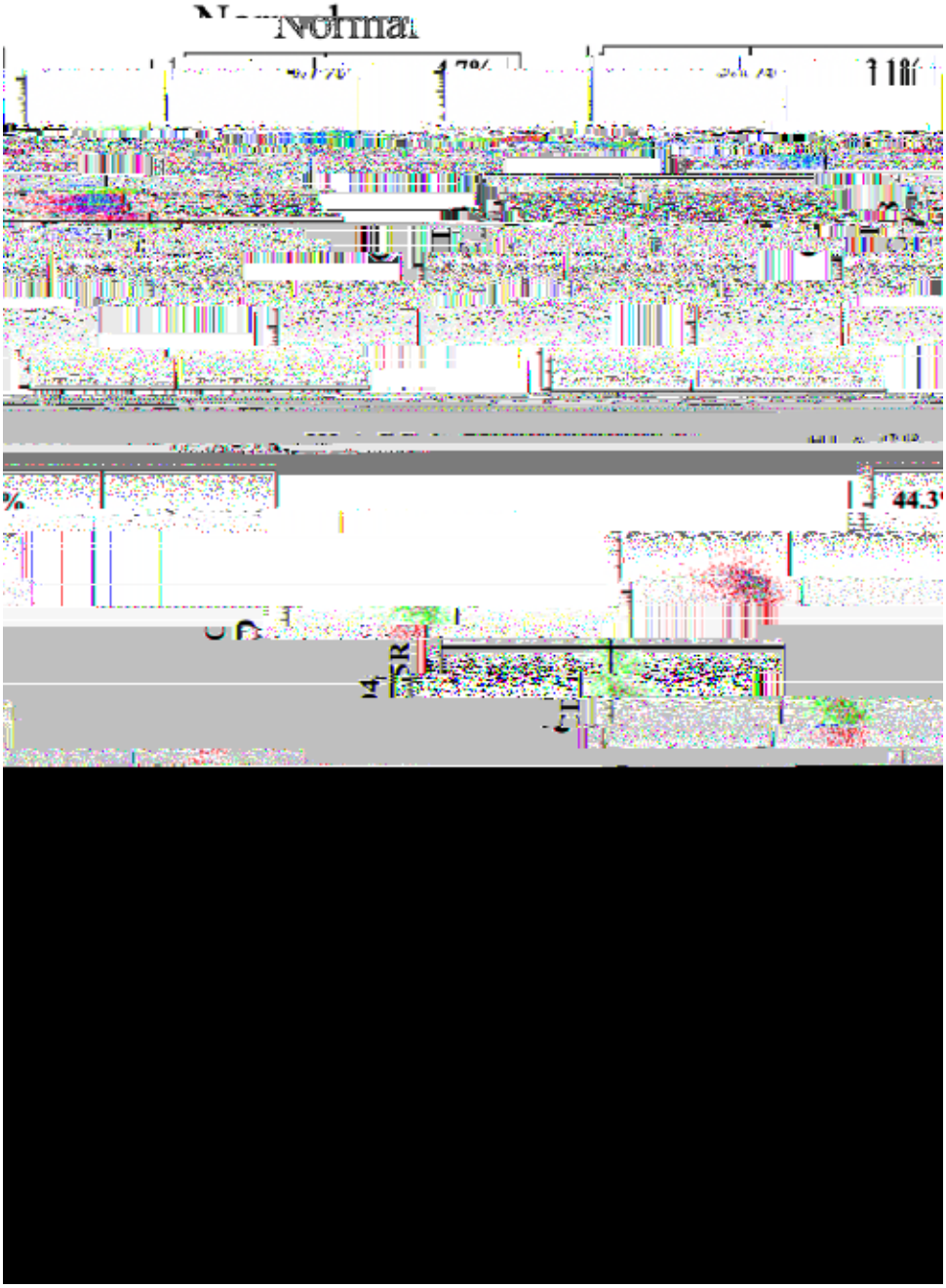


Markers

CD3/CD4/CD8/CD19/ CD16/CD56/CD14/CD45

Primary Immunodeficiency 2 (PID2)

Utility



STAT Gain of Function (SGOF)

Utility

- Tests the phosphorylation of Signal Transducer and Activator of Transcription 1 (STAT1) to determine if there is evidence of enhanced phosphorylation or delayed dephosphorylation
- Useful to determine if variants in STAT1 are gain of function

Clinical indication/general description

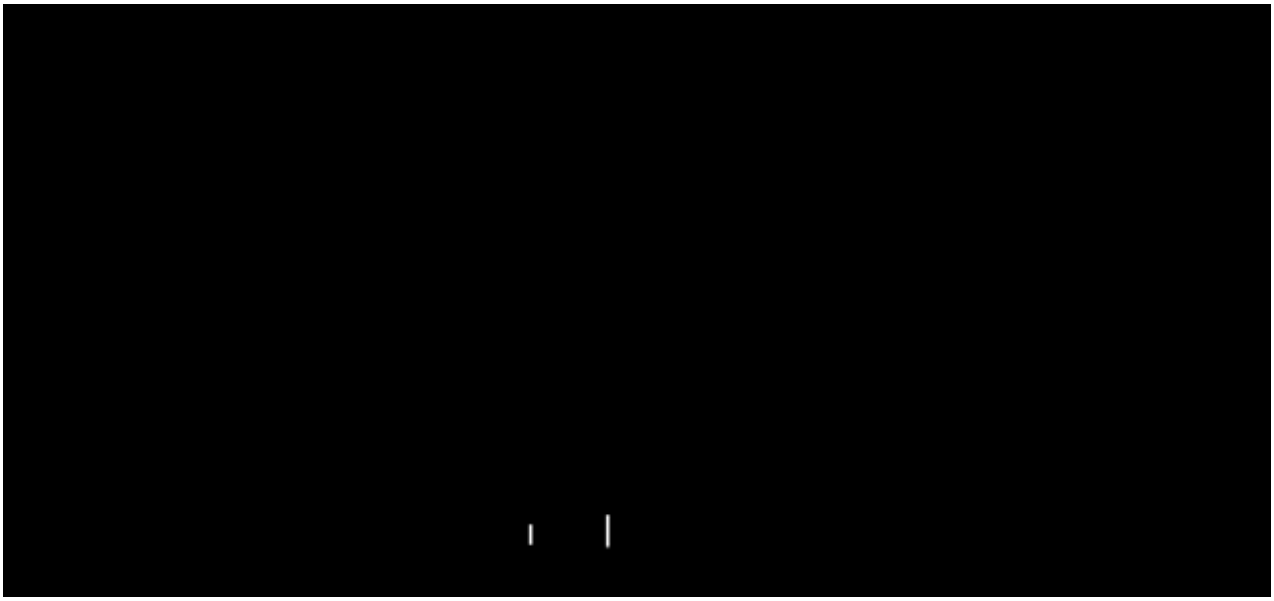
STAT1 is involved in mounting both innate and adaptive immune responses to viruses and bacteria. It can be activated by several ligands, including INF , INF , EGF, PDGF and IL-6. Loss-of-function mutations are associated with susceptibility to mycobacterial, salmonella and herpesvirus infections. Gain-of-function mutations in STAT1 can lead to chronic mucocutaneous candidiasis, other invasive fungal infections and autoimmune disorders such as psoriasis, SLE and alopecia.

Detection methodology

This assay utilizes recombinant IL-6 to activate STAT1 in CD4+ T cells. The cells are stimulated for 15 minutes, washed, rested and analyzed for pSTAT1 expression at 15, 30, 60 and 120 minutes post-stimulation to determine the levels of pSTAT1 and total STAT1 expression.

Markers

CD3/CD4/CD8/CD45RA/CD45RO/CD19/CD16/CD56/CD14/CD45/HLA-DR/pSTAT1/STAT1



T Cell Mitogen (TMITO)/ T Interleukin Proliferation (TINTL)

Utility

- Functional evaluation of T cell proliferation to mitogens

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 7-10 days. Diminished or absent proliferative response to T cell stimuli is consistent with a primary (SCID) or secondary immunodeficiency disease that a□ects T lymphocytes (cellular immunity). This assay tests the proliferative function of T cells to T cell receptor antibodies and a variety of mitogens.

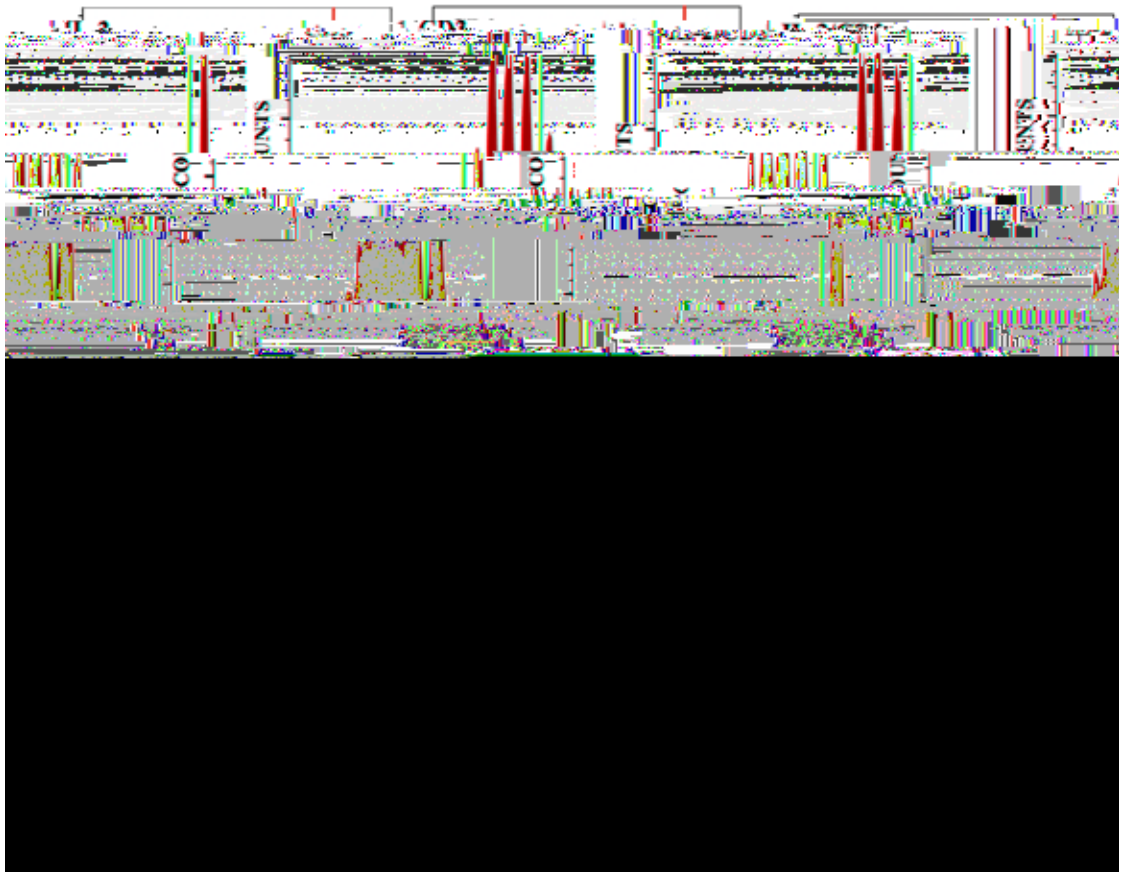
Detection methodology

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.

Markers

CD4/CD8

TINTL: Using the same methodology as above, this assay uses several cytokines to induce growth (IL2, IL7, IL15). This is useful when evaluating for CD25 deficiency. Since all lymphocytes require endogenous IL2 for growth in vitro, in cases of CD25 deficiency, the IL2 response will be abnormal while the IL7 and IL15 responses will be normal.



T Helper IL17 (THIL17)

Utility

- Diagnostic screen for hyper-immunoglobulin E syndrome (HIES)

Clinical indication/general description

Hyper-immunoglobulin E syndrome (HIES), also known as Job syndrome, is characterized by pulmonary infections, staphylococcal abscesses, eczema, and abnormalities of bone and connective tissue. IgE levels are typically very high. HIES can look very similar to severe eczema; thus, a clinical test to be able to di□erentiate these syndromes is clinically useful. The defects in hyper-IgE syndrome are caused by mutations in the transcription factor STAT3. STAT3 is requiiption xsentiattionp5 (cal aeaSe look vgrpic37)10 (er)pclinica5 (cal aeaSeIL)4(r)Tac2 (tr)yiattionkinyndr

Toll-Like Receptor (TLREC)

Utility

- Functional evaluation of toll-like receptors (TLRs)
- Screen for MYD88, IRAK-4, NFKB1A and XIAP

Clinical indication/general description

Toll-like receptors (TLRs) recognize a variety of molecules conserved in microorganisms that are not present in humans, such as lipopolysaccharides in bacteria and double-stranded RNA in viruses. Defects in MYD88 and IRAK-4, molecules required for TLR4 signaling, have been detected in patients with recurrent, invasive pneumococcal or staphylococcal infections. Infants and young children are particularly susceptible to infections when they have defects in TLRs since the adaptive immune system has not developed at this age to o□er protection.

Defects in the X-linked inhibitor of apoptosis (XIAP) protein are associated with the development of X-linked lymphoproliferative syndrome-2 (XLP-2), hemophagocytic lymphohistiocytosis (HLH) and very early onset inflammatory bowel disease (VEO-IBD). XIAP is critical for downstream signaling of nucleotide-binding oligomerization domain-containing protein 2 (NOD2). The interaction of NOD2 with its ligand MDP can be utilized to determine if XIAP deficiency is indicated.

Detection methodology

A functional flow cytometric-based assay tests TLR4 function by assessing the ability of monocytes to produce tumor necrosis factor-alpha (TNF). Peripheral blood is incubated with lipopolysaccharide or L18-Muramyl DiPeptide (NOD2 ligand) and TNF production is measured. This assay detects defects in MYD88 and IRAK-4, and I B . Patients with a NOD2 signaling defect will demonstrate a compromised ability to induce expression of TNF to L18-Muramyl DiPeptide. This assay also detects defects in XIAP by measuring TNF- levels.

Markers

CD14/TNF-

T Regulatory-FOXP3 (TREG)

Utility

- Determine CD4+CD25+FoxP3+ regulatory T cells (TREG) in the peripheral blood
- Diagnostic screen for the presence of immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome by assessing T regulatory cells
- Measures CD25 and CTLA-4 expression

Clinical indication/general description

IPEX is an X-linked recessive disorder causing widespread autoimmune manifestations. Mutations in the forkhead transcription factor FoxP3 are responsible for this disease. FoxP3 is crucial to the development, survival and e□ector function of regulatory T (Treg) cells, a cell population essential to immune regulation. Treg cells account for approximately 5 to 10% of peripheral blood CD4+ T cells. Treg cells also express high levels of CD25, the high a□nity binding alpha subunit of the IL-2 receptor. IPEX-like diseases can also be caused by deficiencies in CD25 or STAT5b, key signal transduction subunits of the IL-2 receptor. Treg cells also express CTLA-4, a known inhibitor of immune response.

Detection methodology

Use of antibodies to CD4, CD25, CD3, CTLA-4 and FoxP3 allows for the determination of the percentage of Treg cells in peripheral blood of patients suspected of IPEX or IPEX-like diseases.

Markers

X-Linked Lymphoproliferative Syndrome (XLP)

Utility

- Evaluate the presence of SLAM-associated protein (SAP) and X-linked inhibitor of apoptosis (XIAP) in peripheral blood
- Diagnostic screen for X-linked lymphoproliferative syndrome (XLP), types 1 and 2
- Detection of carrier status in female relatives of XLP patients

Clinical indication/general description

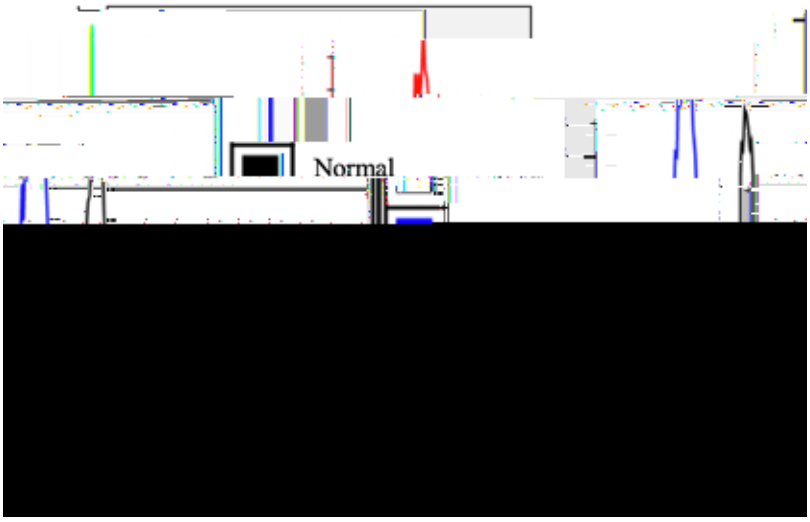
XLP, also called Duncan disease, is a rare X-linked disorder a□ecting predominantly male patients. Mutations in SH2D1A gene encoding the signaling lymphocyte activation molecule (SLAM)-associated protein (SAP) cause XLP1. XLP type 2 (XLP2) is caused by mutations in BIRC4 gene encoding the XIAP protein.

Detection methodology

Intracellular protein expression of SAP and XIAP in peripheral blood lymphocytes is determined by flow cytometry. Deficient expression of SAP and XIAP in lymphocytes is associated with XLP type 1 or XLP type 2, respectively.

Markers

CD3/CD4/CD8/CD45RA/CD45RO/CD19/CD16/CD56/CD14/CD45/SAP/XIAP/HLA-DR



Te _ a e	C de	Diag _ic¯_i¡
Autoimmune Lymphoproliferative Syndrome	AILYMP	• Screen for ALPS
Absolute T4	AT4	• Monitory treatment e□cacy 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
Cytokine IBD	CYTIBD	• Screen for defects in IL-10 receptor • Screen STAT3 function
Cytotoxicity/Apoptosis	CYTAPO	• Screen for hemophagocytic lymphohistiocytosis • Functional evaluation of natural killer cells
Functional Asplenia/Howell-Jolly Body Detection	FAHJB	• Screen for splenic dysfunction
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
LRBA	LRBA	• Screen for LRBA expression
Mendelian Susceptibility to Mycobacterial Diseases	MSMD	• Screen for expression of CD119 and CD212 • Screen for p-STAT1 and p-STAT4 activity • Measure IL-12 production
Neutrophil Function and Phenotype	NPF	• Functional evaluation of neutrophil oxidative burst • Screen for chronic granulomatous disease • Carrier status detection in female relative of CGD
Perforin Granzyme	PERGRA	• Screen for hemophagocytic lymphohistiocytosis • Perforin, granzyme A and granzyme B detection in lymphocytes
PHOX	PHOX	• Assess expression of the NADPH oxidase complex
Primary Immunodeficiency 1	PID1	• General T, B and NK cell evaluation
Primary Immunodeficiency 2	PID2	• Comprehensive screen for cellular and humoral immunity
STAT Gain of Function	SGOF	• Determine functional mutations in STAT1
T Cell Interleukin Proliferation	TINTL	• Functional evaluation of T cell proliferation to interleukins
T Cell Mitogen Proliferation	TMITO	• Functional evaluation of T cell proliferation to mitogens
T Helper IL-17	THIL17	

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