Neuberg DIAGNOSTICS

● India ● UAE ● South Africa ● USA

New INSIGHTS

Primary Immunodeficiency Disorder

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What are Primary Immune Deficiencies (PIDs)?

- PIDs are a group of inherited disorders that affect the development and/or function of the immune system. As per an estimate, 1 in 1200 individuals are affected with a PID. This incidence however is increased in populations with a higher degree of consanguinity. These diseases usually result in recurrent or persisitent severe infections, autoimmunity, auto-inflammation or maligancies.Though not uncommon, they are grossly under-daignosed due to lack of awarness about these diseases.
- While most children with persistent infections may have a normal underlying immune system, it is important to recognize a child with an underlying PID so that further investigations can be performed. Prompt and early diagnosis not only helps initiate appropriate treatment, but also helps further genetic counselling for the family.

Primary Immunodeficiency diseases affect more than

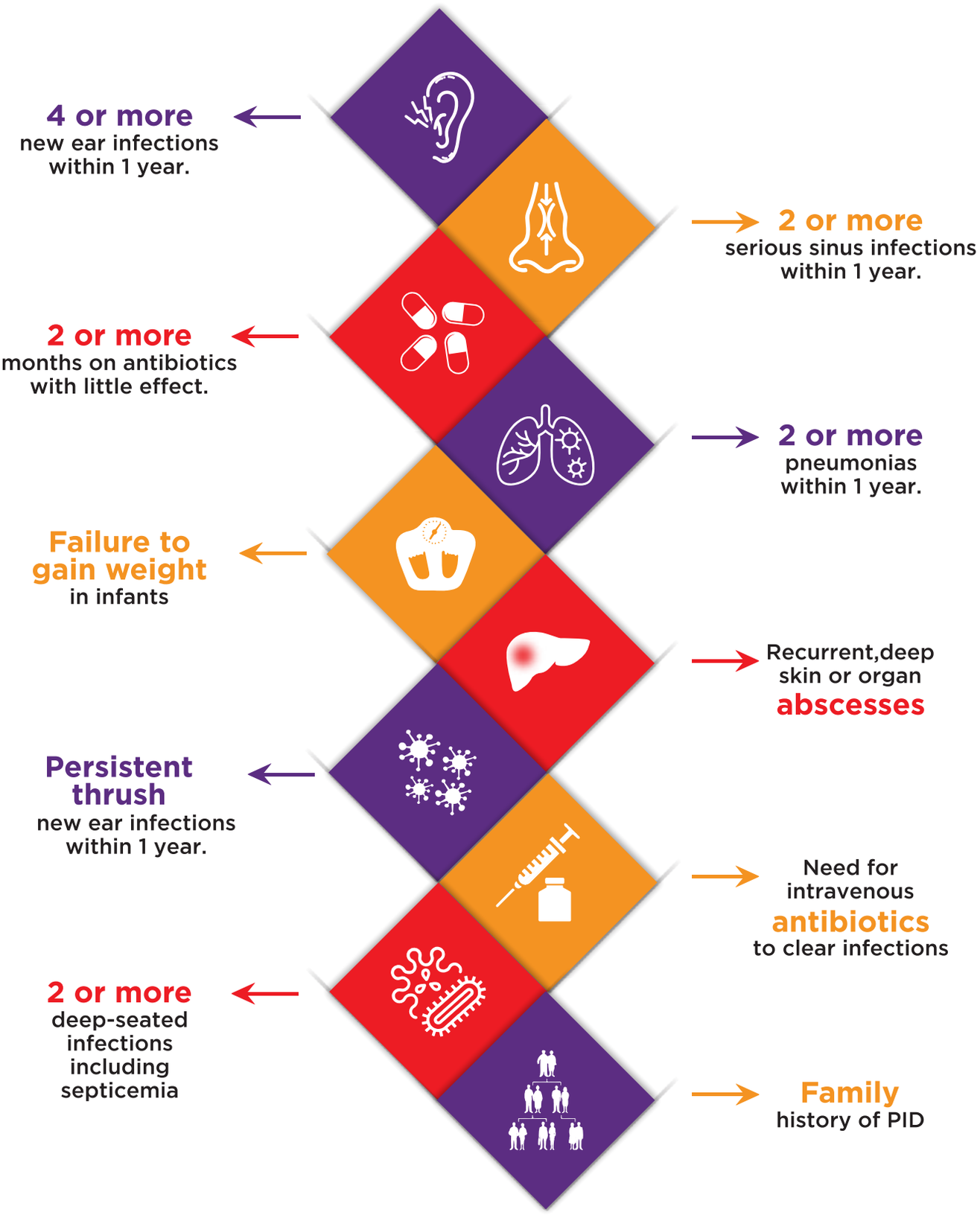
6 Million

people worldwide

With no gender, age or geographical boundaries.

When to suspect PID?

Careful clinical evaluation is crucial for identification of PID. Ten warning signs have been suggested by the European society for Immunodeficiencies (ESID):



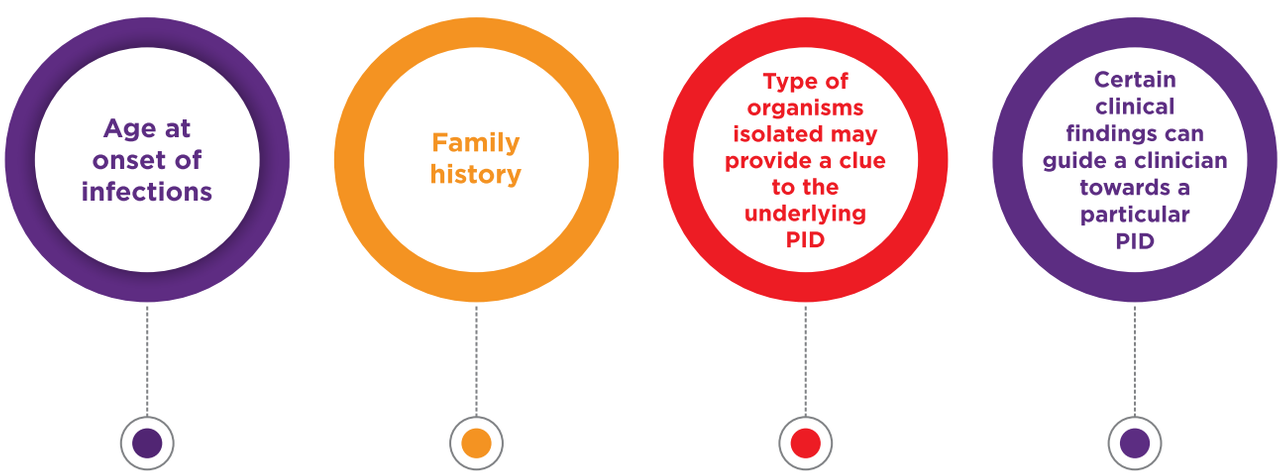
Patients showing these signs and symptoms must be subjected to further evaluation to confirm/exclude a PID.

Common clinical manifestations of the major groups of PIDs include

- **Combined T and B deficiency –** Opportunistic infections (Systemic viral infections, fungal and bacterial infections).
- **Antibody deficiency –** Upper and lower respiratory tract, GI tract, skin infections, sepsis and meningitis. Common organisms- encapsulated bacteria (Pneumococcus, H influenza)
- **Phagocytic defects –** suppurative infections – lung abscess, liver abscess, lymphadenitis, osteomyelitis. Organisms – S aureus, Klebsiella, Aspergillus, Candida, Burkholderia
- **Complement deficiency –** Infections with encapsulated bacteria eg: Meningococcal meningitis.

Clinical approach

Careful clinical evaluation is crucial for identification of PID. Ten warning signs have been suggested by the European society for Immunodeficiencies (ESID):



(a) T cell defects (eg: Severe Combined Immune Deficiency) often present in the first 6 months of life.

(b) B cell defects (eg: X-linked Agammaglobulinemia) present after 6 months of age, once maternally transmitted IgG levels wean off.

PIDs are genetic disorders and a detailed family history is of paramount importance in making a timely diagnosis.

(a) Fungal infections – Aspergillus, Unsual pathogens – Burkholderia, Serratia, Pseudomonas -> Chronic granulomatous disease

(b) Encapsulated bacteria – Pneumococcus, H influenza -> Antibody deficiency (B cell defect), complement deficiency, asplenia

(c) Viral, fungal and bacterial infections -> t cell defect

(d) Atypical mycobacteria – M abscessus, M avium etc -> Mendelian susceptibility to mycobacterial disease

(a) Chronic eczema + Low platelet counts (in a boy)-> Wiskott Aldrich syndrome.

(b) Cold staphylococcal abscess +Eczema + High IgE -> AD hyper IgE syndrome (Jobs syndrome).

(c) Extensive molluscum contagiosum + warts + High IgE -> DOCK8 deficiency.

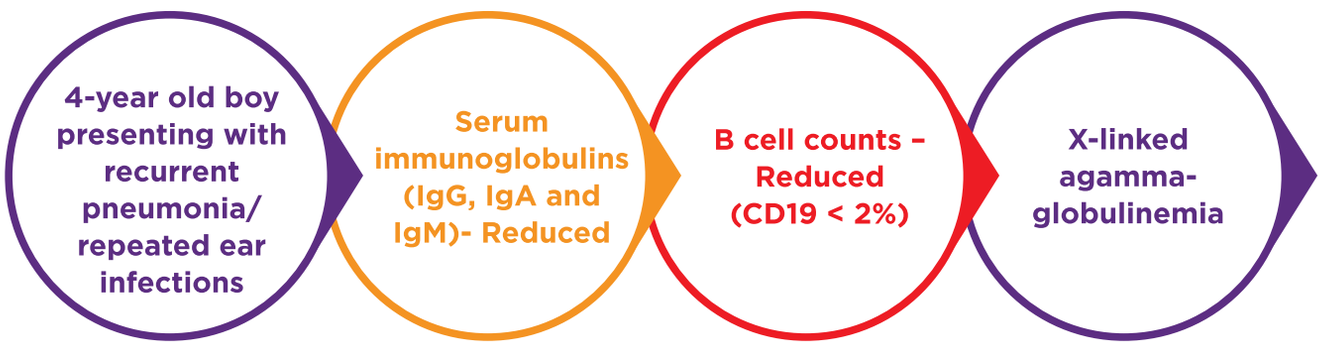
(d) Delayed cord fall in a neonate + very high neutrophil counts -> Leucocyte adhesion deficiency (LAD).

Screening tests for PIDs

Genetic tests are the standard diagnostic methods for most PIDs, but these are time consuming, expensive and not easily available. More easily available screening tests can be used to narrow down the possible diagnosis and then carry out appropriate confirmatory genetic tests.

Example of screening tests:

Case 1



Immunoglobulin profile

Total Immunoglobulin levels

Immunoglobulin-A RATE NEPHELOMETRY	<27 mg/dl	**Neonate (4 Days)** 0-2.2 **Less than 2 yrs** 14-108 **Less than 12 yrs** 29-270 **Adult (20 -60 yrs)** 70-400 **>60 Yrs** 90-410
Immunoglobulin-G RATE NEPHELOMETRY	361 mg/dL	**New born – (4 days)** 700-1480 **Less than 2 yrs** 500-1200 **Less than 12 yrs** 700-1650 **Adult (20 -60 yrs)** 700-1600 **>60 Yrs –** 600-1560
Immunoglobulin-M RATE NEPHELOMETRY	41 mg/dl	**New born – (4 days)** 5-30 **Less than 2 yrs** 43-239 **Less than 12 yrs** 50-260 **Adult (20 -60 yrs)** 40-230 **>60 Yrs –** 30-360

TBNK flow cytometry :

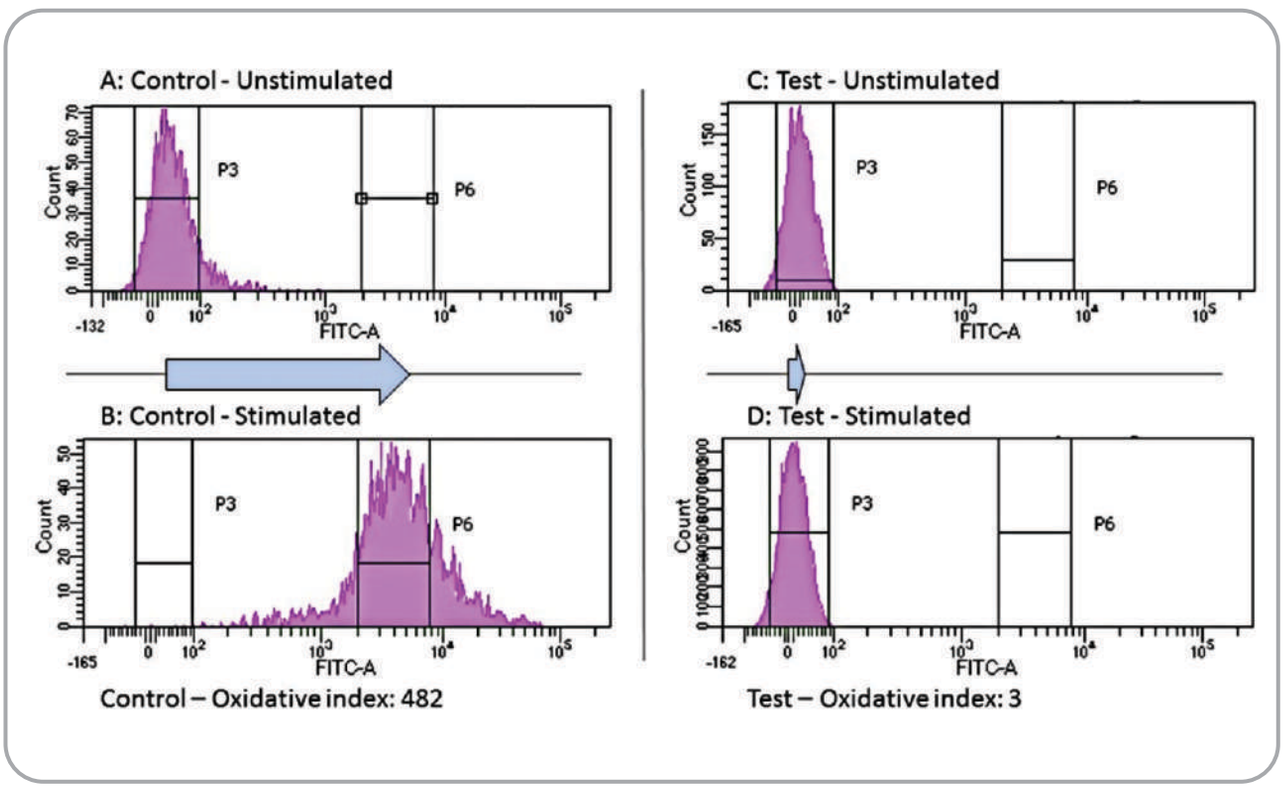
Test Name TBNK (Flowcytometry)	Test Result	Biological Reference range
TBNK- ABSOLUTE LYMPHOCYTE COUNT FLOWCYTOMETRY	4887 cells/microL	**0 to 2 years :** 700-11,9000 **2 to 5 years :** 1700 - 6900 **5 to 10 years :** 1100- 5900 **10 to 16 years :** 100 - 5300 **Adults :** 1000-2800 cells/microL
CD3 + : FLOWCYTOMETRY	92.8%	%
CD19 + : FLOWCYTOMETRY	1.0%	%
CD3 – (CD56 + CD16 +) FLOWCYTOMETRY	5.2%	%
CD3 + (Absolute counts) FLOWCYTOMETRY	4535 cells/microL	**0 to 2 years :** 600-8000 **2 to 5 years :** 900 - 4500 **5 to 10 years :** 700- 4200 **10 to 16 years :** 800 - 3500 **Adults :** 700-2100 cells/microL
CD19 + (Absolute counts) FLOWCYTOMETRY	49 cells/microL	**0 to 2 years :** 600-8000 **2 to 5 years :** 900 - 4500 **5 to 10 years :** 700- 4200 **10 to 16 years :** 800 - 3500 **Adults :** 700-2100 cells/microL
CD3 – CD56 – CD16 + (Absolute counts) FLOWCYTOMETRY	245 cells/microL	**0 to 2 years :** 100-1400 **2 to 5 years :** 100 - 1000 **5 to 10 years :** 90- 900 **10 to 16 years :** 70 - 1200 **Adults :** 90-600 cells/microL

Interpretation : “In view of decreased immunoglobulins and low B cell counts, Possibility of X linked agammaglobulinemia needs to considered

Case 2

- A 2-year old boy presented with repeated episodes of suppurative lymphadenitis. He was admitted with severe pneumonia.
- Blood investigations showed marked neutrophilic leukocytosis and thrombocytosis. He was suspected to have chronic granulomatous disease and evaluated further.

DHR test: A transport control sample was sent from an unrelated donor.



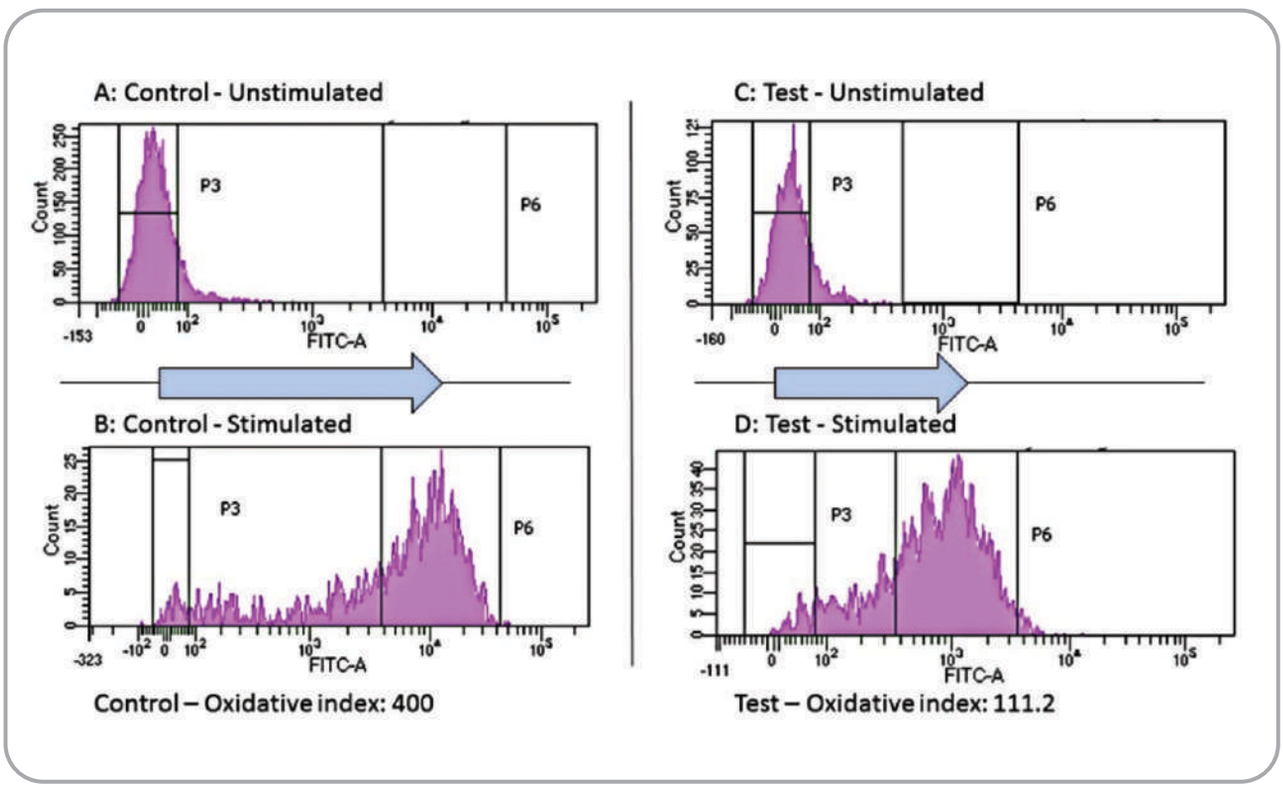
Legend: A and B are graphs from a transport control sample. A clear shift is seen in the neutrophil fluorescence post PMA stimulation, indicated by the blue arrow. In C and D which represent the test sample, there is hardly any shift in the fluorescence post PMA stimulation.

Interpretation: "Neutrophil oxidative burst is markedly reduced in comparison with the control sample. In view of history and clinical features, consistent with **chronic granulomatous disease"**

Case 3

- A 9-month-old girl with chronic diarrhea and pyoderma gangrenosum. She had a delayed cord fall at birth.
- Initial work-up showed marked neutrophilic leukocytosis. Clinical suspicion of Leucocyte Adhesion defect and Chronic granulomatous disease.

DHR test:



CD18 Flow cytometry for Leucocyte Adhesion defect:

CD18 expression on neutrophils – Control : **99.8%**

CD18 expression on neutrophils – Test: **0.1%**

Interpretation: "Almost complete absence of CD18 expression on neutrophils, consistent with Leucocyte Adhesion defect. Neutrophil respiratory burst appears preserved."

Test/Profile	Components	Methodology	DoS Code	Sample Requirements	Reporting Time
CD18 [For screening Leucocyte adhesion deficiency]	enumeration of CD18	Flow cytometry	C0201	3 ml of whole blood in EDTA tube (Lavender top) & Sodium heparin. Ship refrigerated	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Dihdrorho-damine (DHR)	oxidative burst	Flow cytometry	D0029	3 ml (2 ml min.) whole blood in a 1 Green Top (Sodium Heparin) tube, ship refrigerated. DO NOT FREEZE	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Double Negative T- cells (DNT)	Analysis of double negative T - cells	Flow cytometry	D0031	Collect 3 ml whole blood in EDTA tube (Lavender top). Ship refrigerated	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
CD 19	CD 19	Flow cytometry	F0088	3 mL (2 mL minimum) whole blood in 1 EDTA tube (Lavender Top) and 3 mL (2 mL minimum) whole blood in 1 Sodium Heparin tube (Green Top) or 2 mL (1 mL minimum) heparinized Bone marrow. Ship Immediately at 18-22°C. DO NOT REFRIGERATE OR FREEZE. Specify time, date and clinical details on test request form.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
CD 20	CD 20	Flow cytometry	F0091	3 mL (2 mL minimum) whole blood in 1 EDTA tube (Lavender Top) and 3 mL (2 mL minimum) whole blood in 1 Sodium Heparin tube (Green Top) or 2 mL (1 mL minimum) heparinized Bone marrow. Ship Immediately at 18-22°C. DO NOT REFRIGERATE OR FREEZE. Specify time, date and clinical details on test request form.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
CD4/CD8 absolute counts	CD3/4/8 & 45	Flow cytometry	10001	Sample to be shipped at room temperature 48 hrs of collection	Sample reaching the lab before 2 PM on weekdays will be reported on the same day by 7 PM

Test/Profile	Components	Methodology	DoS Code	Sample Requirements	Reporting Time
Immuno-globulin IgA, Serum	IgA in Serum	Nephelometry	10118	2 mL (1 mL min.) serum from 1 SST. Fresh sample preferred. Ship refrigerated.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Immuno-globulin IgE, Serum	IgE in Serum	Chemilumine-scence	10119	2 mL (1 mL min.) serum from 1 SST. Fresh sample preferred. Ship refrigerated.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Immuno-globulin IgG, Serum	IgG in Serum	Nephelometry	10120	2 mL (1 mL min.) serum from 1 SST. Fresh sample preferred. Ship refrigerated.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Immuno-globulin IgM, Serum	IgM in Serum	Nephelometry	10121	2 mL (1 mL min.) serum from 1 SST. Fresh sample preferred. Ship refrigerated.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Nitroblue Tetrazolium (NBT)	Tetrazolium nbt	Manual	N0022	3 mL (2 mL min.) Whole blood in 1 Green Top (Sodium Heparin) tube, ship refrigerated. DO NOT FREEZE	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
Primary immuno deficiency disorders (PIDs) Panel	NBT, TBNK, IgG, IgA, IgM, IgE, DHR, CD3, CD4, Cd8	Flow cytometry	P0087	For Patient- 2 mL (1 mL min.) serum from 1 SST & 2 mL (1 mL min) blood from 1 Green Top (Sodium Heparin) & 2 mL (1 mL min) Whole Blood Lavender Top (EDTA) tube. For control - 2ml whole blood in green top (Sodium heparin) from a normal control preferably unrelated. Ship refrigerated	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM
TBNK	T Cell, B Cell & Natural Killer Cell enumeration	Flow cytometry	T0066	3 mL (2 mL min.) Whole blood in 1 Lavender Top (EDTA) tube. Ship immediately at 18 - 22°C. DO NOT REFRIGERATE OR FREEZE. Specify time, date and clinical details on test request form.	Sample reaching the lab before 1 PM on weekdays will be reported on the same day by 7 PM

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