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AMPATHCHAT

Diagnosing PID: The Genetic Revolution

PATHOLOGISTS · PATOLOË
Drs Du Buisson, Kramer, Swart, Bouwer Inc./Ing.
AMPATH

Please contact your local Ampath pathologist for more information.

Primary immunodeficiencies (PID) are a diverse group of inborn errors in immunity. PID can present at any age, with the more severe forms presenting in infancy. Due to the heterogeneity of presentation and age of onset, the diagnosis of PID is often delayed or missed. There are at least 300 known genetic defects resulting in PID.

Clinical history is paramount in the initial diagnosis, particularly when infections are:

- **S**evere (requiring hospitalisation or intravenous antibiotics)
- **P**ersistent (patients never recover completely)
- **U**nusual microorganisms or sites of infection
- **R**ecurrent
- **R**un in the family

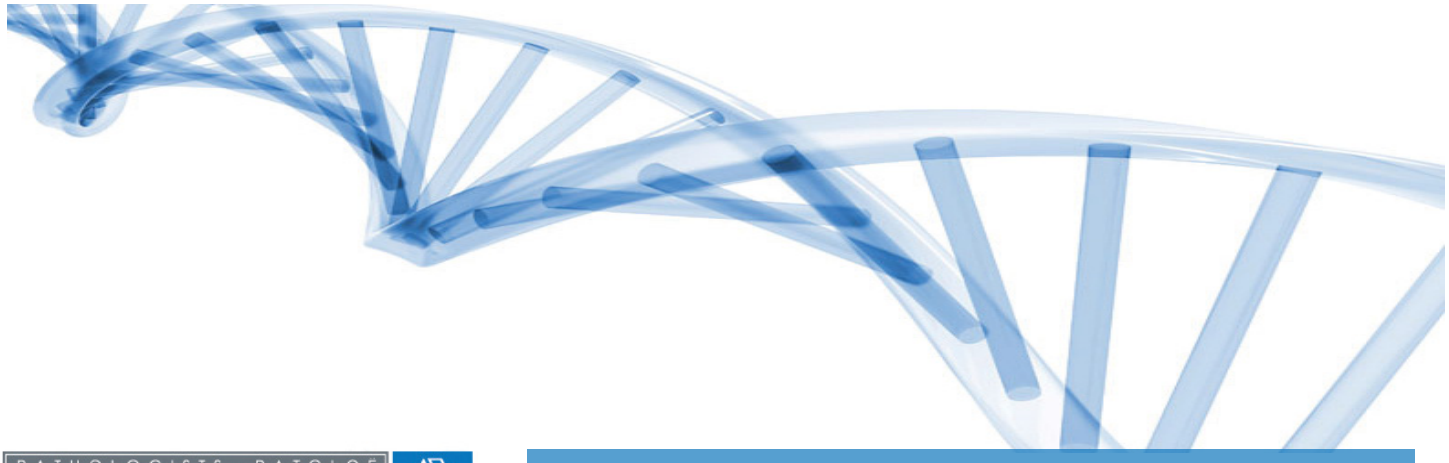
A genetic diagnosis is crucial for the diagnostic confirmation of PID. In addition, a genetic diagnosis can be used to screen family members, for prenatal testing and to further understand the mechanism of disease and treatment options.

To facilitate the genetic diagnoses of PID, Ampath has developed a local PID panel that utilises next-generation sequencing (NGS) technology. The Ampath PID panel includes 99 of the most important PID genes and covers the following immunodeficiency diseases:

- Severe combined immunodeficiency (SCID)
- Hyper IgM syndrome
- Hyper IgE syndrome
- Immune dysregulation
- Common variable immunodeficiency (CVID)
- Lymphoproliferative syndromes, including autoimmune lymphproliferative syndrome
- Chronic granulomatous disease (CGD)
- MHC Class I and MHC Class II deficiencies
- Anhidrotic ectodermal dysplasia with immunodeficiency
- Autoimmunity with lymphoproliferation
- Antibody deficiencies
- T-regulatory cell defects
- Familial haemophagocytic lymphohistiocytosis (FHL), including FHL with hypopigmentation
- Chemokine signalling defects
- Innate immunity defects
- Complement deficiencies
- Isotype deficiencies
- Thymic defects with congential abnormalities

What testing should be performed if PID is suspected?

If a clinician suspects PID, initial testing of the immune system should be performed. A stepwise approach to the diagnosis of PID is recommended to ensure that the most cost-effective and clinically relevant testing is performed.



Please contact your local Ampath pathologist for more information.

A STEPWISE APPROACH TO THE LABORATORY DIAGNOSIS OF PID		
	Test	**Association**
First-line investigations	Exclude HIV	
	Assessment for atopy	As indicated on history
	Full blood count: • Differential count for neutrophil and lymphocyte numbers • Platelet count and morphology	Lymphopaenia is an important indicator of possible severe combined immunodeficiency (SCID) Small platelets seen in Wiskott-Aldrich syndrome
	Serum immunoglobulin (Ig) G, M, A and E	IgE should be tested in patients who may be at risk of hyper IgE syndrome.
	Cystic fibrosis (CF) screening	Suspected on history and clinical examination
Second-line investigations	Specific antibody response: • Targeted to polysaccharide-specific antigens (pneumococcus and *H. influenzae*) and protein antigens (tetanus, diphtheria and *H. influenza*)	Indicated with recurrent bacterial infections, even in the presence of normal immunoglobulin. Please note that patients have to be off immunoglobulin replacement therapy for six months. If these antibody levels are decreased, the patient should be revaccinated and antibody responses should be repeated four weeks after vaccine boosting to determine an appropriate increase in specific antibody responses. An unconjugated pneumococcal vaccine, e.g. Pneumovax®, should be given to determine an appropriate polysaccharide antigen response.
	Lymphocyte subsets: • B-cell numbers (CD19) • T-cell numbers (CD3). T-helper (CD4) and T-suppressor (CD8) cells should also be measured. • Natural killer cells (CD16 and CD56)	B-Cells: Absent in XLA-linked agammaglobulinaemia when all immunoglobulin isotypes are severely reduced. T-Cells: Reduced in T-cell defects, combined immunodeficiencies and occasionally CVID. NK-Cells: Isolated NK-cell deficiencies may be associated with recurrent herpes virus infections.
	Neutrophil function: • Neutrophil oxidative burst • Neutrophil studies for leukocyte adhesion, chemotaxis and phagocytosis	CGD Leukocyte adhesion defects
	Total haemolytic complement: • Classic • Alternative	Complement deficiencies
Third-line investigations	Lymphocyte proliferation studies: • Response to mitogens, e.g. PHA, PMA, PMA +ionomycin, anti-CD3, anti-CD3+IL2 or to recall antigens, e.g. Candida, tetanus, varicella	T-cell deficiencies, including SCID, chronic mucocutaneous candidiasis
	Neutrophil antibodies	Auto-immune neutropaenia
	Lymphocyte maturation panel: naive and memory T-cells	Diagnosis of SCID and combined T-cell defects
	Recent thymic emigrants (T-cells)	Very low in SCID Can be used to monitor bone marrow regeneration post transplant
	Memory B-cells	Memory B-cells categorise subsets of CVID patients
	Alpha/beta, gamma/delta T-cell receptor type	Abnormal in leaky SCID, hypomorphic SCID, T-cell defects with oligoclonality.
	TRECs and KRECs	Used for neonatal screening for SCID and XLA on blood monospots – useful to do prior to giving live vaccines at birth.
Fourth-line investigations	• Genetic/molecular studies • Cytokine studies • Enzyme studies • T-regulatory cells • Th 17 cells • Surface markers for X-linked SCID • BTK (diagnosis of XLA)	Consult an Immunology pathologist. Tel: 012 - 678 0613/4

Adapted from M. Esser, B. Eley, M.S. Suchard, S. Buldeo and C. van Rooyen.
TREC: T-cell receptor excision circles; KREC: Kappa receptor excision circles; SCID: Severe combined immunodeficiency; BTK: Brutons tyrosine kinase; CGD: Chronic granulomatous disease; XLA: X-linked agammaglobulinaemia; PMA: Phorbol myristate acetate; CD3: Cluster of differentiation 3; IL2: Interleukin 2; CVID: Common variable immune deficiency; PHA: Phytohaemagglutinin

When to request a Ampath genetic PID panel

Genetic testing can be used as the next line of testing to make or confirm the diagnosis of PID. A genetic diagnosis is very important when a patient has a severe immunodeficiency that may require bone marrow transplantation or other invasive therapies. It is crucial when other family members and future pregnancies may be affected.

How to request the Ampath PID panel

Specimen type: One full EDTA tube (test code: **PID**).

All patients referred for PID genetic testing should be discussed with an immunology pathologist before testing. Genetic counselling is also required before and after testing.

The Ampath PID panel has an estimated cost of R17 600 and includes 99 of the most relevant genes involved in PID. Please contact the laboratory for a quote, as prices may vary for different medical aids. *Prices quoted are valid until December 2016.*

The estimated turnaround time for the Ampath PID panel is six weeks, provided that all relevant clinical and laboratory information is readily available. Each patient's results are discussed individually, taking into account all laboratory findings and the clinical and family history.

Other genetic tests for single gene defects or the testing of family members are available on request.

For more information or for test requests:

Ampath Immunology: 012 678 0613/4

Ampath genetic counsellor: 012 678 1362/50