

# Primary immunodeficiencies

## Diagnosis of primary immunodeficiencies



## List of some common abbreviations

CBC	Complete blood count
CVID	Common variable immunodeficiency
CGD	Chronic granulomatous disease
Ig (e.g. IgG)	Immunoglobulin (e.g. immunoglobulin G)
IPOPI	International Patient Organisation for Primary Immunodeficiencies
LAD	Leukocyte adhesion deficiency
PID	Primary immunodeficiency
SCID	Severe combined immunodeficiency
WAS	Wiskott-Aldrich syndrome
XLA	X-linked agammaglobulinaemia

Primary immunodeficiencies — Diagnosis of primary immunodeficiencies (1<sup>st</sup> edition).  
December 2012

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Published by IPOPI: [www.ipopi.org](http://www.ipopi.org)

## Introduction

**This booklet explains how primary immunodeficiencies (PIDs) are classified and diagnosed.**

PIDs are a large group of different disorders caused when some components of the immune system (mainly cells and proteins) do not work properly. The immune system normally helps the body to fight off infections by micro-organisms such as bacteria, viruses or fungi. People with PIDs are therefore more prone than other people to catching infections.

The immune system is divided into 'innate' and 'adaptive' (or 'acquired') immune systems.

- The **innate immune system** comprises various types of cells that react immediately to invading micro-organisms regardless of whether the body has encountered them before. These cells include 1) phagocytic cells (e.g. neutrophils and macrophages) that recognise, swallow and kill invading micro-organisms; 2) other leucocytes (e.g. eosinophils, basophils and mast cells) that release agents that produce inflammation and which are toxic to invading micro-organisms; and 3) natural killer (NK) cells that kill infected body cells.
- The **adaptive (acquired) immune system** acts like a memory — when exposed to a new molecule it recognises as foreign (an 'antigen'), it builds a specific immune response that can be quickly activated if the body encounters that antigen again. The main cells involved are the T cells and B cells (also known as T and B 'lymphocytes'). T cells attack invading micro-organisms inside the body's own cells and produce chemicals called cytokines, which help to gather and organise other immune cells. B cells produce immunoglobulins (or 'antibodies'), which kill specific micro-organisms and help the phagocytic cells to work.

These different cells interact and work together in various ways (and with other components such as 'complement', discussed below) to fight off infections and also to protect against cancers. There are well over two hundred PIDs classified according to the part of the immune system that is defective (see table).

PIDs can be identified in children or in adults. In some cases, patients have lived with the symptoms for years before the correct tests were done to diagnose their PID. It is crucial that doctors consider the possibility of a PID in paediatric or adult patients with relevant symptoms, and that they perform the appropriate tests. Some simple tests are available to most doctors, provided that they are aware of PIDs. Other, more detailed, tests are usually done by a doctor who specialises in the immune system (an immunologist). This booklet explains the main tests used.

PID classification <sup>1</sup>	Examples of PIDs
Combined T and B cell deficiencies	<ul style="list-style-type: none"> <li>• Severe combined immunodeficiency disorder (SCID)</li> <li>• Complete DiGeorge syndrome</li> <li>• CD40 and CD40L deficiencies<sup>2</sup></li> </ul>
Well-defined syndromes with immunodeficiency	<ul style="list-style-type: none"> <li>• Wiskott-Aldrich syndrome (WAS)</li> <li>• Ataxia telangiectasia</li> <li>• Hyper immunoglobulin E syndrome</li> </ul>
Predominantly antibody deficiencies	<ul style="list-style-type: none"> <li>• Common variable immunodeficiency disorders (CVID)</li> <li>• Various immunoglobulin (Ig) deficiencies, e.g. X-linked agammaglobulinaemia (XLA, or 'Bruton disease') and selective IgA deficiency</li> <li>• CD40 and CD40L deficiencies<sup>2</sup></li> </ul>
Diseases of immune dysregulation	<ul style="list-style-type: none"> <li>• Lymphoproliferative syndromes</li> <li>• Familial haemophagocytic lymphohistiocytosis</li> <li>• CD24 deficiency</li> </ul>
Congenital defects of phagocyte number or function, or both	<ul style="list-style-type: none"> <li>• Defects in neutrophil differentiation (e.g. severe congenital neutropenia, X-linked neutropenia)</li> <li>• X-linked chronic granulomatous disease (CGD)</li> <li>• Leukocyte adhesion deficiency (LAD)</li> </ul>
Defects in innate immunity	<ul style="list-style-type: none"> <li>• Several molecular defects predisposing to viral infection (e.g. herpes encephalitis) and fungal infections (e.g. chronic candidiasis)</li> <li>• Anhidrotic ectodermal dysplasia with immunodeficiency</li> </ul>
Auto-inflammatory diseases	<ul style="list-style-type: none"> <li>• Familial Mediterranean fever</li> <li>• TNF receptor-associated period syndrome (TRAPS)</li> </ul>
Complement deficiencies	<ul style="list-style-type: none"> <li>• Various complement deficiencies</li> </ul>

<sup>1</sup>Classification of International Union of Immunological Societies, 2011 (see 'Further information and support').

<sup>2</sup>CD40 and CD40L deficiencies are classified in both categories. TNF, tumour necrosis factor  $\alpha$ .

## Overview of PID diagnosis

PIDs are usually first suspected because of infections. A PID might be suspected in children or adults who have infections that are unusually common, severe or persistent, or if they are caused by unusual microorganisms. The types of infections that occur can give a clue to which type of PID is present.

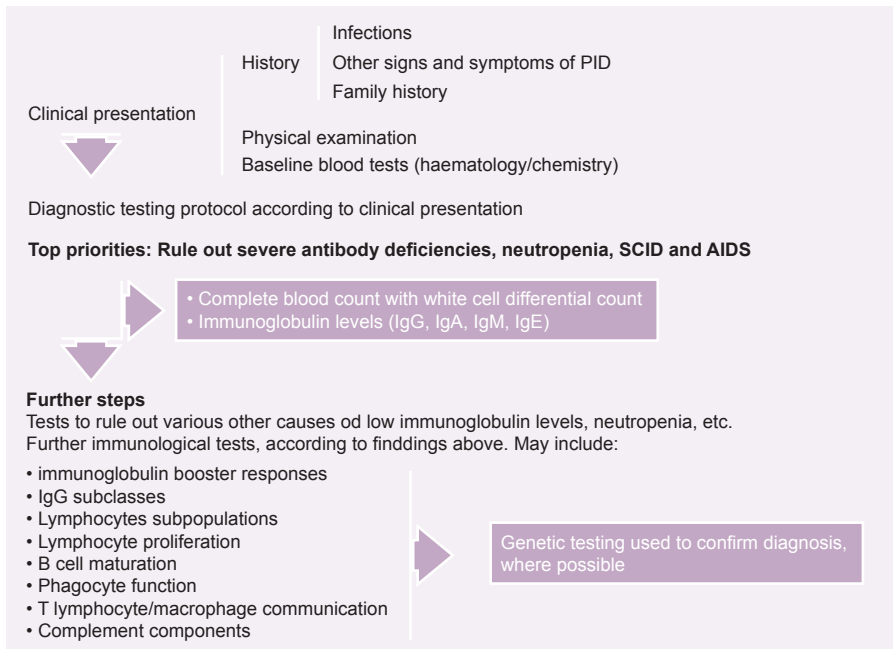
PIDs can also cause the body to attack itself — this is called ‘auto-immunity’.

This can cause various symptoms, including pain and swelling in the joints (‘arthritis’), skin rashes and a loss of red blood cells (‘anaemia’). Some severe PIDs cause signs that can be seen soon after birth. For example, complete DiGeorge syndrome can cause facial malformations, heart disease and problems with the nervous system that are present at birth. A family history of PIDs or PID-like symptoms, and basic blood tests, can also give useful information.

Based on these findings, further tests of the immune system are performed (see figure). These are done in a stepwise way to first rule out the most severe kinds of PID, e.g. severe combined immunodeficiency (SCID). The most important of these tests are:

- Complete blood count (CBC) with a differential count of the white blood cells.
- Measurement of immunoglobulin levels.

The following sections discuss these tests, and additional tests that may be performed.



Based on European Society for Immunodeficiencies protocol, 2011 (see ‘Further information and support’).

## Blood cell tests

### Complete blood count (CBC)

Normal blood contains many different kinds of cells, many of which are involved with the immune system. The CBC shows how many of each kind of cell are present in a small sample of a patient's blood. For PID diagnosis, this needs to include a 'differential' count of the various types of white blood cells (together known as 'leukocytes') present. The CBC results are compared with the 'reference' range of values normally found in healthy people.

Available to most doctors, the CBC is a crucial test because it reveals any severe defects in the blood that could be caused by a PID. For example, patients with SCID, one of the most serious forms of PID, typically have very low levels of T cells. SCID is a medical emergency as infants with the condition are at high risk of life-threatening infections. Early diagnosis and therapy are essential to improve the patient's chance of survival. The International Patient Organisation for Primary Immunodeficiencies (IPOPI) is leading efforts to establish routine screening for SCID among new-born infants in Europe. Ataxia telangiectasia is another PID associated with reduced T cell levels, although in this disorder there is a progressive decrease in these cells over time.

SCID is a medical emergency.

The CBC also detects 'neutropenia', a low level of neutrophils that can be caused by many PIDs (e.g. severe congenital neutropenia and X-linked neutropenia). The CBC can also identify problems with the platelet cells, which normally help the blood to clot. Patients with Wiskott-Aldrich syndrome (WAS) have too few platelets and hence are at risk of bleeding. The CBC also detects anaemia, an abnormally low level of the red blood cells that carry oxygen to the tissues of the body.

These various types of blood cells can be affected by many different diseases and drugs, as well as by PIDs. The results of the CBC must therefore be interpreted carefully, taking into account whether any of these factors are present. In addition, as the immune system develops during childhood, the levels of cells must be interpreted according to the age of the patient.

The CBC gives a vital indication of problems with the immune system, but it is not sufficient alone to diagnose a PID. Rather, the CBC guides the use of more detailed tests on specific types of cells. Normally these tests are performed by a specialist immunologist, where one is available.

## Other blood cell tests

**Lymphocyte subpopulation and proliferation tests:** Lymphocytes (i.e. T and B cells) can be divided into various subpopulations, e.g. helper T cells (also called 'CD4' cells) and cytotoxic T ('CD8') cells. A count of the different kinds of lymphocytes in the blood can help identify which PID is present. In addition to counting how many lymphocytes there are, it is also important to test how well they work. For example, certain tests identify how well these cells multiply (or 'proliferate') when they are stimulated to do so by chemicals or antigens that normally trigger an immune response.

**Granulocyte function tests:** Neutrophils, eosinophils, basophils and mast cells are together known as 'granulocytes'. Normally these cells (mainly neutrophils) produce hydrogen peroxide (sometimes called 'reactive oxygen') to kill bacteria and fungi. The amount of hydrogen peroxide produced is usually measured using a laboratory test called the dihydrorhodamine (DHR) oxidative burst test. This is an important diagnostic test for the PID known as X-linked chronic granulomatous disease (CGD), in which the neutrophil count is normal (or high), but these cells do not work properly. Other tests are used to assess how well these cells migrate toward an attractant (this is called 'chemotaxis') and how effectively they kill and swallow bacteria.

**B cell maturation tests in bone marrow:** This test is used to diagnose PIDs called agammaglobulinaemias, such as X-linked agammaglobulinaemia (XLA, also known as Bruton disease). These conditions are caused by genetic defects that prevent B lymphocytes from developing properly. Normally, mature B lymphocytes produce immunoglobulins. Patients with these disorders therefore have very low levels of immunoglobulins (see 'Immunoglobulin measurements'), as well as low levels of B cells.

**Cell protein expression:** Tests can identify deficiencies in certain proteins that are normally found on the surface of white blood cells. For example, CD40 and CD40L are two proteins that together allow T helper cells to stimulate an immune response by B cells. CD40 or CD40L deficiencies are severe PIDs also known as hyper-immunoglobulin M disorders. Defects in CD11 and CD18 proteins can cause leukocyte adhesion deficiency (LAD).

**Switched memory B cells:** Memory B cells are a group of B cells that can 'remember' antigens the body has fought before. When stimulated by that antigen again, the memory B lymphocytes 'switch' on to produce antibodies against that antigen. The measurement of switched memory B cells can be useful in investigating common variable immunodeficiency disorders (CVID), hyper-IgE syndrome, and CD40/CD40L deficiencies.

# Immunoglobulin measurements

## Immunoglobulin levels

Immunoglobulins (or antibodies) are proteins that recognise infecting micro-organisms and help immune cells to destroy them. Most PIDs cause the body to produce too few immunoglobulins, or none at all. The measurement of immunoglobulins G (IgG), A (IgA), E (IgE) and M (IgM) is therefore very important in PID diagnosis.

Different PIDs have characteristic effects on immunoglobulins (see table). For example, patients with XLA (Bruton disease) have low levels of all immunoglobulin classes. Selective IgA deficiency, as its name suggests, reduces only IgA. Patients with isolated IgG subclass deficiency have normal levels of total IgG, but reduced levels of one or more particular types (or 'subclasses') of IgG — measurement of these subclasses helps to diagnose this condition.

Example PID	IgG	IgA	IgE	IgM
Ataxia telangiectasia	Subclasses may be low	Often low	Often low	High (monomers)
XLA (Bruton disease)	Low	Low	Low	Low
CD40/CD40L deficiency	Low	Low	Low	Normal or high
CVID disorders	Low	Low		May be low
Hyper-IgE syndrome	Normal	Normal	High	Normal
IgG subclass deficiency	Total normal, one or more subclass low	Normal	Normal	Normal
SCID	Usually low	Usually low	Usually low	Usually low
Selective IgA deficiency	Normal	Low or absent	Normal	Normal
WAS		Often high	Often high	Low

CVID, common variable immunodeficiency disorders; SCID, severe combined immunodeficiency; WAS, Wiskott-Aldrich syndrome; XLA, X-linked agammaglobulinaemia.



Some PIDs (e.g. those classified as diseases of immune dysregulation) do not affect immunoglobulin levels. Therefore, if immunoglobulin levels are normal this does not necessarily mean that the patient does not have a PID.

Importantly, as immunoglobulin levels change during childhood, the results of these tests must be interpreted according to the age of the patient.

## Antibody response to vaccines

Vaccines are produced using micro-organisms that are killed or inactivated using specific methods. Normally, the vaccine triggers the production of antibodies against this micro-organism and therefore prepares the body to fight off a future infection.

Some PIDs cause this type of immunity to fail. For example, patients with several PIDs do not produce a normal increase in IgG in response to 'polysaccharide' vaccines such as influenza type B vaccine or Streptococcus pneumonia (or 'pneumococcal') vaccine. These PIDs include WAS, anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) and specific antibody deficiency with normal immunoglobulin concentrations and normal numbers of B cells.

For more information about the use of vaccines in patients with PID, see the IPOPI booklet 'Stay healthy! A guide for patients and their families'.

When diagnosing PIDs, doctors measure antibody levels (or 'titres') 3–4 weeks after giving a vaccine in order to test whether the immune system responds properly. As well as polysaccharide vaccines, the response to protein vaccines (such as tetanus and diphtheria vaccines) is also tested. These responses must be interpreted according to the age (in the case of children) and according to what vaccines a person has received in the past.

## Complement proteins

Complement is the term used to describe a group of serum proteins that kill micro-organisms and assist other immune cells. A group of PIDs, called complement deficiencies, are caused by defects in this system. Specific tests are used to detect complement deficiencies. The main complement test is called the CH50 assay. A separate test called the AH50 assay measures a particular type of complement.

## Tests for other conditions

Other diseases and certain drugs can sometimes cause signs and symptoms similar to those of PIDs. The following tests may be performed to rule out these causes before a diagnosis of a PID is made.

- **Human immunodeficiency virus (HIV):** The HIV virus attacks the immune system and this can lead to the condition known as acquired immunodeficiency syndrome (AIDS). The HIV test is a simple and widely available blood test.

PIDs are genetic conditions that are mostly present at birth. They are not related to HIV/AIDS, which is caused by the HIV virus, and they cannot be 'caught' or spread.

- **Autoimmune diseases:** Autoimmune symptoms can be caused by various conditions and hence blood tests to exclude these conditions may be performed. For example, the presence of rheumatoid factor in the blood is tested to exclude rheumatoid disease.
- **Cancer screening:** Tests are often used to check for cancer in patients with symptoms suggestive of PID. Medical imaging may be used, for example a magnetic resonance imaging (MRI) or positron emission tomography (PET) scan of the lymph glands.



## Genetic testing

PIDs are caused by defects in the genes (that is, the portions of DNA) that are involved in the development and workings of the immune system. The exact genetic defects that cause some PIDs are known. These include SCID, CGD, hyper-IgE syndromes, WAS, XLA and complement defects. These defects are mostly inherited from the parents, but others can arise through genetic mutations that happen during pregnancy. A careful family history can provide important information in the diagnosis of PIDs.

By analysing a patient's DNA, doctors can identify any defects present and thereby confirm the diagnosis of a specific PID. This is called genetic testing and it can also:

- Help in decisions about PID treatment, including replacement of the defective gene in those countries where this is being performed.
- Predict how the PID will progress and affect the patient in the future (the 'prognosis').
- Check if an unborn foetus has a genetic defect that causes a PID (so-called 'prenatal' testing).
- Help in counselling adults with a PID who wish to have children. PIDs are inherited via a number of complex patterns. Ideally, people with PID who wish to have children should seek genetic counselling so that they are aware of the risks of passing on their PID to their children.

Genetic testing can only be done by specialised laboratories. It is not available in all countries. National PID patient organisations can provide further information about availability of genetic testing in specific countries.

While genetic testing is very useful for diagnosing many PIDs, little is known about the genetic basis of some conditions (such as CVID disorders).



# Primary immunodeficiencies

## Further information and support

This booklet has been produced by IPOPI. Other booklets are available in this series. For further information, and details of PID patient organisations in 43 countries worldwide, please visit [www.ipopi.org](http://www.ipopi.org). Two notable sources for detailed information are:

The 2011 international classification of PIDs, published free online as:  
Al-Herz W, et al. Primary Immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Frontiers in Immunology* 2011; vol. 2: article 54

The 2001 PID screening protocol published by the European Society for Immunodeficiencies and colleagues: De Vries E, et al. Patient-centred screening for primary immunodeficiency, a multistage protocol designed for non-immunologists: 2011 update. *Clinical and Experimental Immunology* 2012; vol. 167: p. 108–119



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Supported by an educational grant from Octapharma.