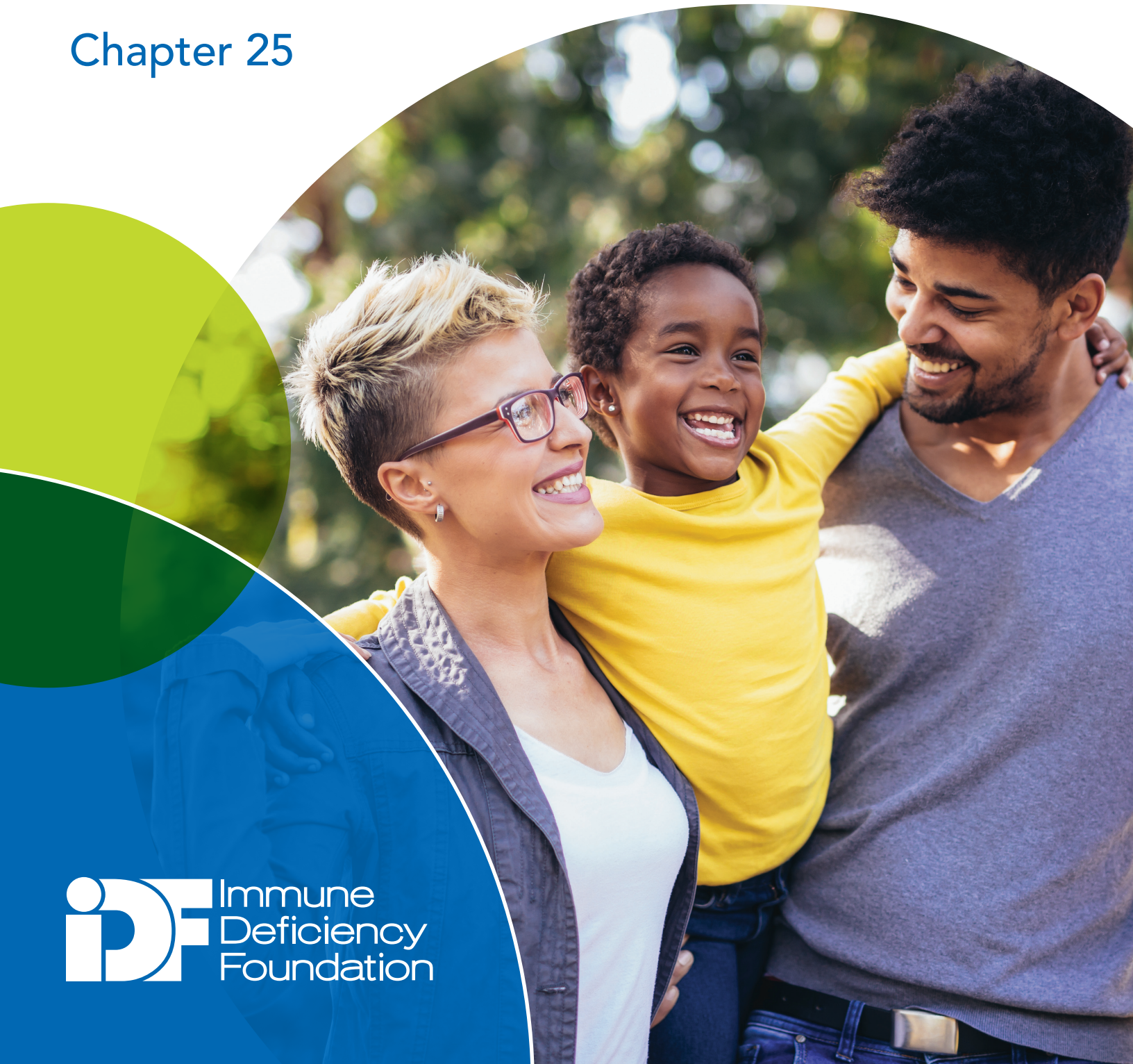
Immune Deficiency Foundation

Patient & Family Handbook

For Primary Immunodeficiency Diseases

Chapter 25



Immune Deficiency Foundation

Immune Deficiency Foundation

Patient & Family Handbook

For Primary Immunodeficiency Diseases

6th Edition

The development of this publication was supported by Shire, now Takeda.

Immune Deficiency Foundation

110 West Road, Suite 300
Towson, MD 21204
800.296.4433

www.primaryimmune.org
idf@primaryimmune.org

Chapter 25
Laboratory Tests

James Verbsky, MD, PhD, Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Laboratory studies are necessary to determine the presence of a primary immunodeficiency disease (PI). This is usually prompted by an individual experiencing some clinical problems, particularly recurrent and/or chronic infections. Information regarding the types of organisms, the sites of infection, and the therapies required to treat the infections often help focus the laboratory studies. The individual's medical history and physical exam direct the appropriate choice of laboratory tests.

Normal vs. Abnormal Laboratory Values

Proper interpretation of any laboratory value depends on comparison of the result to the appropriate established normal reference ranges that in some cases are age-specific. To determine what is normal, the laboratory test is performed on a group of healthy individuals, usually adults and equally divided between males and females. These results are used to determine what the normal range is, using a variety of statistical approaches. A common statistical measurement is called a 95% confidence interval, which is the range that includes 95% of the results from normal tested subjects (as in a bell curve). It is important to note that when the definition of the normal range is set as a 95% confidence interval, 5% of the selected normal population will fall in the abnormal range (both high and low), even though they were originally selected as being normal. This is one of the challenges with using statistical methods to define a normal range and must be remembered when evaluating a test result falling near either end of the normal range.

Using the measurement of height as an example, normal individuals can be just above or just below a normal range (or 95% confidence interval) and still be normal. Someone 1 inch taller than the 95% confidence interval is not necessarily a giant, and someone 1 inch shorter is not necessarily a short person. In fact, by definition, 2.5% of normal individuals will be below the 95% confidence limit, and 2.5% will be above. The fact that 5% of otherwise normal healthy individuals will fall outside the normal range is important when looking at laboratory results—finding a value outside of the reference range does not automatically represent an abnormality. The clinical relevance of an abnormal laboratory finding must be based on the clinical history as well as the size of the difference from the normal range.

Characteristics of the group, such as the age of the group, used to determine the normal range are crucial since the immune system undergoes substantial development during infancy and childhood. The range of test values that are normal in infancy will be quite different when the child is 2 or 20 years old.

Consequently, all studies in children must be compared with age-matched controls. If the laboratory reporting test results does not provide age specific information, it is important to consult with a specialist who knows the age-specific reference ranges. Optimally, the laboratory doing the test should provide this, but if unavailable, there are published age-specific reference ranges.

The most common laboratory tests used to evaluate immune disorders are used to identify:

1. Antibody deficiencies
2. Cellular (T cell) defects
3. Neutrophil disorders
4. Complement deficiencies

These four major categories of tests for PI are described hereafter. Another emerging laboratory test for the diagnosis of PI is genetic testing. In the past, genetic tests were available mainly in

research settings, but now, several commercial labs offer genetic sequencing for the identification of known mutations causing various types of PI.

Antibody Deficiency (or Humoral Immune Function)

The standard screening tests for humoral immune function starts with measurement of immunoglobulin (Ig), or antibody, levels in the blood serum. These consist of IgG, IgA, IgM, and sometimes IgE levels. The results must be compared to age-matched controls.

There are also tests for specific antibody production. These tests measure how well the immune system can make antibodies against vaccines, as a marker of how well the antibody arm of defense is functioning. There are two main pathways of antibody production being tested, the T cell dependent pathway measured by the antibody response to protein antigens, such as tetanus and diphtheria toxoids, and the T cell independent pathway measured by the antibody response to carbohydrate antigens, such as those found in the pneumococcal polysaccharide vaccine (PPSV) known as PNEUMOVAX. In this approach, the person is immunized with these common vaccines, and blood samples are obtained immediately prior to and approximately four weeks after the immunization to evaluate how well the individual forms specific antibodies.

In some instances, the person may have already been immunized with these vaccines as part of their normal care and will already have circulating antibodies (if they make antibodies), while in other instances the individual may have little or no specific antibody prior to the immunization. The use of different types of vaccines is necessary because certain people with recurrent infections (and normal or near normal immunoglobulin levels) have been identified with an abnormality in the response to carbohydrate antigens but a normal response to protein antigens. (See Selective Immunoglobulin Deficiency: IgA and IgM Chapter.)

It is worth noting that during the maturation of the immune system, the response to carbohydrate antigen vaccines lags behind the response to protein antigen vaccines. (This is the reason for having a childhood version of the pneumococcal vaccine, the PCV13, which makes it easier for infants to respond to Streptococcus pneumoniae). The interpretation of vaccine responses is best done by an allergist/immunologist provider who deals with individuals with PI on a regular basis.

The ability to evaluate the antibody response in a person already receiving Ig replacement therapy is more difficult. This is because Ig is rich in most of the specific antibodies that are generated following immunizations. When immunized with common vaccines, it is difficult to tell the difference between the antibody provided by the Ig replacement therapy and any that might have been made by the individual. The solution to this is to immunize with vaccines that are not normally encountered by the general population and therefore are unlikely to be present in Ig preparations. Uncommon vaccines, such as typhoid or rabies vaccine, can serve this purpose.

It is important to note that in a person with a previously confirmed defect in antibody production, stopping therapy to recheck for antibody levels and immunization response is unnecessary and may place the individual at risk of acquiring an infection during the period when the Ig replacement therapy is stopped. In someone whose diagnosis of an antibody immunodeficiency is unclear, however, it may be necessary to stop Ig replacement therapy for a period of four to six months so that the individual's humoral immunity can be adequately assessed.

Additional studies used to evaluate people with antibody deficiencies include measuring the different types of lymphocytes in the blood using a test called flow cytometry. The B cell is the lymphocyte that has the ability to produce antibodies. B cells may be absent in certain immune disorders associated with antibody, such as X-linked Agammaglobulinemia (XLA). This test can also evaluate the ability of the B cells to mature.

In addition, analysis of DNA can be used to confirm a particular diagnosis, such as the gene encoding Bruton tyrosine kinase (BTK) associated with XLA. Finally, there are studies done in specialized laboratories to assess Ig production by cultured lymphocytes in response to a variety of different kinds of stimuli.

Cellular (T cell) Immunity

The laboratory evaluation of cellular or T cell immunity focuses on determining the numbers of different types of T cells and evaluating the function of these cells.

The first test to evaluate for T cell immunity occurs without most people knowing about it, as all states in the U.S. now screen for very low T cells numbers at birth. This occurs through the newborn screening program, a program to detect severe treatable genetic defects in otherwise healthy looking infants. All newborns have a blood sample drawn, obtained through a heel prick, that is sent to the state laboratory where a screening test called the TREC assay is performed. This measures the number of T cells in the blood at birth, and is an excellent way to screen for severe deficiencies of T cells, as can be seen in Severe Combined Immunodeficiency (SCID). If this is abnormal, an immunologist in the state is contacted and further testing is recommended. As of December 2018, all newborns born in the U.S., including all 50 states, Washington, DC, and Puerto Rico, are screened for severe T cell immunodeficiency. This newborn screening should make the successful treatment of SCID and other related severe T cell immunodeficiencies easier since infants with these conditions will be identified at birth and appropriate treatment, such as hematopoietic stem cell transplantation (bone marrow transplantation) or gene therapy, can be readily undertaken. (See Newborn Screening Chapter.)

Outside of the newborn period, the simplest test to evaluate possible decreased or absent T cells is a complete blood count (CBC) and differential to establish the total blood (absolute) lymphocyte count. This is a reasonable method to assess for diminished T cell numbers, since normally about three-quarters of the circulating lymphocytes are T cells and a reduction in T cells will usually cause a reduction in the total number of lymphocytes, or total lymphocyte count. One must be careful, however, as individuals can have fairly normal absolute lymphocyte count but still have a significantly low T cell count. The actual T cell count can be confirmed by using flow cytometry with markers specific for different types of T cells.

The measurement of the number of T cells is often accompanied by cell culture studies that evaluate T cell function. This is done by measuring the ability of the T cells to respond to different types of stimuli, including mitogens (such as phytohemaglutinin [PHA]) and antigens (such as tetanus toxoid, candida antigen). The T cell response to these various stimuli can be measured by observing whether the T cells divide and grow (called proliferation) and/or whether they produce various proteins important in immune responses called cytokines (such as interferon). There are an increasing variety of functional tests that are available to evaluate T cells. An immunologist is the best person to undertake this interpretation.

Many types of PI are associated with specific genetic defects. This is particularly true of SCID in which more than 20 different genetic causes have been identified. These can all be evaluated using current technology for mutation analysis, and this is the most accurate means to establish the definitive diagnosis.

Neutrophil Function

The laboratory evaluation of the neutrophil begins by obtaining a series of white blood cell counts (WBC) with differentials. The WBC and differential will determine if there is a decline in the absolute neutrophil count (neutropenia). This is the most common abnormal laboratory finding when an individual presents with a clinical history that suggests defective neutrophil immunity. Usually more than a single CBC and differential is necessary to diagnose neutrophil problems.

A careful review of the blood smear is important to rule out certain diseases that are associated with abnormalities in the structure of the neutrophil, or the way it looks under the microscope. Sometimes a bone marrow biopsy is needed to see if the neutrophils are being made properly. If these initial screening tests of neutrophil numbers were normal, testing would then focus on two possible types of PI: Chronic Granulomatous Disease (CGD) and Leukocyte Adhesion Deficiency (LAD). Both of these disorders have normal or elevated numbers of neutrophils, and each of these disorders has distinctive clinical features that can help to direct the appropriate evaluation.

Laboratory testing to diagnose CGD relies on the evaluation of a critical function of neutrophils that kills certain bacteria and fungi—the creation of reactive oxygen. This process, called the oxidative burst, can be measured using a number of different methods. Currently, the most commonly used and most reliable test uses flow cytometry to measure the oxidative burst of activated neutrophils using a specific dye (dihydrorhodamine 123 or DHR), referred to as the DHR test. The DHR test has been used for more than 20 years, and it is extremely sensitive in making the diagnosis. This test can also be helpful in identifying carriers of CGD. (See Inheritance Chapter.) Because of its excellent performance, this test has become the standard in most laboratories supporting clinics that see individuals with CGD regularly. In the past, a dye reduction test called

the Nitroblue Tetrazolium (NBT) test was used but this test had greater variability in its interpretation. The best confirmation of the specific type of CGD is suggested by the results of the DHR test, but it requires confirmation by either specifically evaluating for the defective protein involved or its related gene mutation underlying the disease.

Laboratory testing for the most common form of LAD Type 1 involves flow cytometry testing to determine the presence of a specific protein on the surface of neutrophils (and other leukocytes). When this protein is absent or significantly decreased, the movement of neutrophils to sites of infection is hampered and produces a large increase in the number of these cells in the circulation as well as an increased susceptibility to bacterial skin, oral, and other infections.

Laboratory testing for the most common form of LAD Type 1 involves flow cytometry testing to determine the presence of a specific protein on the surface of neutrophils (and other leukocytes). When this protein is absent or significantly decreased, the movement of neutrophils to sites of infection is hampered and produces a large increase in the number of these cells in the circulation as well as an increased susceptibility to bacterial skin, oral, and other infections.

Complement Deficiency

The standard screening test for deficiencies in the complement system is the total hemolytic complement assay or CH50. In situations with a defect in one complement component, the CH50 will be almost completely negative. Specialized complement laboratories can provide additional testing that will identify the specific complement component that is defective. There are some extremely rare conditions in which there are defects in another complement pathway (the alternate pathway). These can be screened for by using a functional test directed specifically at this pathway, the AH50 test. The complement cascade can also be initiated by the mannose-binding lectin pathway, and there are some individuals with a deficiency in mannose binding lectin, although the clinical relevance of the laboratory finding is inconclusive. (See Complement Deficiencies Chapter.)

Innate Immunity

Laboratory tests are also available to measure the function of the various elements of innate immunity. This includes determining the number and activity of lymphocytes, such as natural killer cells, as well as the function of various cell surface receptors such as the toll-like receptors.

Genetic Testing

Genetic testing (mutation analysis) has become increasingly common in the diagnosis of PI in recent years and is likely to continue to expand in its application as the testing becomes more affordable. These tests allow for the rapid screening for mutations in hundreds of genes that affect the immune system. Some tests are arranged in panels that screen for well-known disease causing mutations while other more in depth tests can look at the genetic code in more detail and uncover variations in immune genes that may be of significance but whose purpose may not be fully known. This data must be interpreted by a provider with experience in the analysis of this complex data set. Many healthy people have genetic changes in these genes, since the variant must be in the right place to affect function of the gene. This technology is already being used in clinical medicine and is rapidly advancing.

Summary

Laboratory testing plays a central role in the evaluation of the immune system. All results must be compared to age-appropriate reference ranges. An accurate medical history, family history, and physical examination are critical in developing the best strategy for laboratory evaluation. This typically begins with screening tests, followed by more sophisticated (and costly) tests chosen based on the initial test results. The range of laboratory testing available to evaluate the immune system continues to expand. This has been driven in part by the recognition of new clinical syndromes associated with recurrent and/or chronic infections.

The direct link between clinical findings and laboratory testing has extended our understanding of PI. The continuation of this trend and laboratory testing of the future will likely be even more sophisticated and help provide further answers to the underlying basis of the expanding range of PI.

Adapted from: Chapter 21 Laboratory Tests. IDF Patient & Family Handbook for Primary Immunodeficiency Diseases 5th Edition. 2013.

The development of this publication
was supported by Shire, now Takeda.

110 West Road, Suite 300
Towson, MD 21204

800.296.4433
www.primaryimmune.org
idf@primaryimmune.org