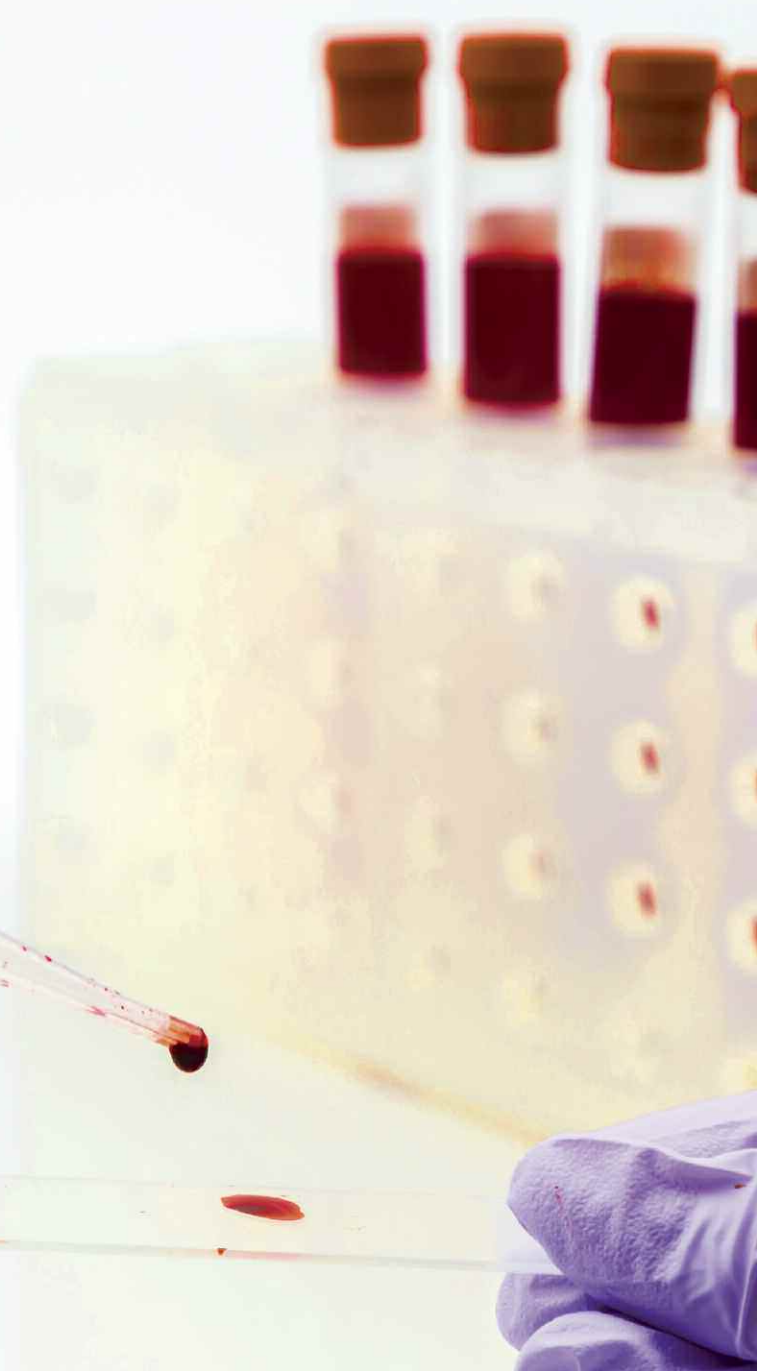


# Routine Primary Immunodeficiency Lab Reports: *What Do They Mean?*

By Bob Geng, MD, MA

**IN THE FIELD** of clinical immunology, laboratory studies as well as symptom presentation are often the keys to accurate diagnosis. There are a myriad of laboratory studies that evaluate the various aspects and functions of the immune system, and it is often confusing for the patient to navigate or understand the legion of labs that are performed and how they are relevant to the care of their condition. Therefore, this article explains the significance of some of the most frequently drawn labs in the area of primary immunodeficiency (PI) evaluation. The following is not an exhaustive list of all the labs that exist in the field of clinical immunology. Rather, they are the labs that almost any patient who is being evaluated for the potential of PI would receive at some point during their care. Understanding these labs will help patients become more knowledgeable members of their own healthcare team.



## Overview of the Immune System

In humans, the immune system is divided between the innate and adaptive systems. Almost all multicellular organisms have evolved to possess some form of innate immunity. It is called innate immunity because it exists in the body without the initial recognition or interaction with any foreign elements. The key to innate immunity is that it works quickly to neutralize any invading foreign elements such as bacteria or viruses. The agents involved in innate immunity include both cells such as neutrophils, macrophages and natural killer cells, as well as molecules in the

blood such as complement proteins. These agents are programmed from birth to recognize key patterns that are common in foreign invaders and danger signals to the body, and will act to neutralize those elements upon recognition.

The limitation of the innate immune system is that it does not acquire memory of the interactions with foreign invaders, and the response is not specific. Therefore, in humans, it cannot exist in isolation and must work in conjunction with the adaptive immune system to provide the body with optimal protection. The adaptive immune system is so named because it adapts to the interactions it has with the specific encounters with foreign invaders. It possesses immunologic memory so that while the initial response may be slow to develop, subsequent responses will be accelerated and augmented. The responses are specifically tailored to the particular foreign invader that is threatening the host at a particular time. Another key element of the adaptive immune system is that it has the capability of generating an

innumerable diverse array of specific responses to the foreign invaders, and it constantly evolves to produce more targeted and stronger responses to those foreign elements. Unlike the innate immune system that recognizes only a limited number of key patterns on foreign elements, the adaptive immune system can learn and evolve to recognize specific patterns unique to each foreign invader.

## THE MOST COMMON LABORATORY EVALUATIONS FOR THE DIAGNOSIS OF PI ARE THE STUDIES THAT EXAMINE THE FUNCTION OF HUMORAL IMMUNITY.

The adaptive immune system is comprised of both cellular components and humoral elements. The main cells that are involved are the B and T cells, which are collectively referred to as lymphocytes. The T cells are then subdivided into helper T cells and killer T cells. The helper T cells assist members of the innate immune system and B cells to fight off infections. The killer T cells directly destroy cells in our body that have been infected with viruses. B cells produce molecules called antibodies that help neutralize foreign invaders or toxins, and these antibodies can mediate direct destruction of foreign invaders, as well as increase the efficiency of the innate immune system to clear infections. These antibodies are divided into several classes: IgG (most abundant), IgA (involved in mucosal immunity), IgM (the initial antibody response) and IgE (involved in allergic disease).

### Humoral Immunodeficiency Labs

The most common laboratory evaluations for the diagnosis of PI are the studies that examine the function of humoral immunity. Humoral immunity encompasses the arm of the immune system that is primarily composed of antibodies, or immunoglobulins (for the purpose of this article these two terms will be used interchangeably). Humoral immunodeficiencies are also the most common form of PI. They can present at birth such as Bruton's agammaglobulinemia or in adulthood such as common variable immunodeficiency (CVID).

The most common humoral immunodeficiency lab is the quantitative immunoglobulin panel that includes an evaluation of IgG, IgM and IgA (see Table 1). The reference ranges for these immunoglobulins are age-specific before age 6 years, when adult levels are generally reached. In order to not overcomplicate the discussion, we will focus on the reference ranges for ages 6 and above. For IgG, the normal range can vary between 700 and 1,500 mg/dL. For IgM, the normal range can vary between 40 and 270 mg/dL. For IgA, the normal range can vary between 80 and 420 mg/dL. For complete agammaglobulinemia (absence of immunoglobulins) due to Bruton's agammaglobulinemia or autosomal recessive agammaglobulinemia, the levels are nearly absent for all types. For CVID, there have to be two types of immunoglobulins that are deficient by at least two standard deviations below the lower limit of normal, and IgG has to be one of those two types (i.e., low IgG and IgA or low IgG and IgM). There can be many other reasons for low quantitative levels of immunoglobulin including hyper-IgM syndrome, IgA deficiency, selective IgM deficiency, combined immunodeficiency syndromes, protein-losing enteropathy, kidney disease (nephrotic syndrome) or medication-induced (i.e., chronic use of immunosuppression drugs).

## PI LABS ARE OFTEN DIFFICULT TO INTERPRET FOR BOTH PATIENTS AND PROVIDERS.

In some cases, the quantity of immunoglobulin may appear normal, but the quality of the immunoglobulin may not be normal. The quality of immunoglobulins is assessed by looking at specific antibody titers. The body may be producing immunoglobulins, but it may not be producing the functional type that helps fight off infections effectively. The most common specific antibody titer that is checked is the pneumococcal titer. Streptococcal pneumonia is one of the most common organisms that leads to upper and lower respiratory infections in adults and children. The most thorough assays assess the levels of antibody made to 23 of the most common virulent subtypes of Streptococcal pneumonia bacteria. The absolute cutoff used for the protective level is 1.3 micrograms/mL for any of the subtypes. Generally, the pneumococcal titers are either checked as part of the routine evaluation for CVID or as

part of the evaluation for specific antibody deficiency, which is a condition in which the quantitative immunoglobulin level is normal, but the patient is still suffering from recurrent infections due to lack of production of specific antibodies. The criterion for an abnormal assay is different depending on the age group assessed. For 2- to 5-year-olds, greater than 50 percent of the 23 subtypes should exhibit protective levels (>1.3 micrograms/mL). For patients older than 6 years, greater than 70 percent of the 23 subtypes should be protective.

Prior exposure is necessary in order to have protective levels of antibody against a certain subtype of pneumococcal bacteria. If someone has never been infected by a strain of pneumococcus, then there may not be a baseline protective level. Therefore, if the baseline level is not adequate, it does not necessarily mean that the patient has a specific antibody deficiency, but that there was no prior exposure. In that situation, the unconjugated polysaccharide vaccine (Pneumovax) will be administered, and the patient will be retested in four to six weeks to determine whether there would be adequate response. If the repeat pneumococcal titers are still inadequate (i.e., less than 50 percent for 2- to 5-year-olds or less than 70 percent for those older than 6 years), then a diagnosis of selective antibody deficiency can be made. However, there is a group of individuals who have a particular deficiency due to inadequate response to polysaccharide antigens (non-protein-based components of the bacteria). To evaluate for that condition, the protein-conjugated pneumococcal vaccine (Pneumovax) can be administered for repeat testing in four to six weeks to determine whether the subtypes covered by Prevnar show protective titers.

Some providers occasionally evaluate the quantitative levels of IgG subclasses to determine whether there is a selective subclass deficiency. There are four subclasses of IgG: IgG1, IgG2, IgG3 and IgG4. IgG1 is the most abundant, making up over 60 percent to 70 percent of the total IgG, and IgG4 is the least abundant and sometimes even undetectable in some normal individuals. Therefore, in the setting of a normal total IgG level, selective subclass deficiency is generally due to a low IgG2 or IgG3 level. These subclasses perform slightly different functions.

**Table 1.**  
**Normal Reference Range for Immunoglobulins in Adults**

Immunoglobulin Class	Concentration in mg/dL
IgG	700–1,500
IgM	40–270
IgA	80–420

**Table 2. Normal Reference Range for Flow Cytometry Lymphocyte Counts**

Lymphocyte Subset	Concentration: Cells/Microliter
CD3	1,000–2,200
CD4	530–1,300
CD8	330–920
CD19	110–570
CD16/CD56	70–480

It is believed that IgG1 and IgG3 are antibodies that focus on recognizing protein and toxin elements of foreign infectious agents. A significant amount of IgG2 antibodies are thought to recognize polysaccharide components of bacteria. The value of checking for subclass deficiency is questionable, and the clinical relevance of a subclass deficiency in isolation is very controversial. Oftentimes, patients with isolated subclass deficiency in the setting of normal total immunoglobulin levels and normal protective antibody titers are asymptomatic, and should not be labeled as being immunodeficient.

### Assessment of Cellular Immune Function

To assess cellular immunity, the most common lab aside from a complete blood count is the basic B and T cell flow cytometry panel (see Table 2). This is a technique that analyzes the number of cells in distinct groups based on their size, presence of specific cell surface markers and degree of granularity inside the cells. The immune cells are often distinguished based on the presence or absence of specific cell surface proteins. The key components of this study are the counts for the CD3, CD4, CD8, CD19 and CD16/CD56 cells (CD stands for cluster of differentiation, and all these components are specific cell surface markers). CD3 is the hallmark of all T cells, including both helper and killer T cells ranging between 1,000 and 2,200 cells/microliter in adults. The reference normal levels are much higher in infants and young children. The ranges are approximates and can differ between institutions and processing laboratories. CD4 is the marker for T helper cells and can range between 530 and 1,300 cells/microliter in adults. CD8 is the marker for T killer cells and can range between 330 and 920 cells/microliters in adults. In normal individuals, the number of CD4 cells always outnumbers the absolute CD8 cells. CD19 is one of the key markers of B cells and can range between 110 and 570 cells/microliter in adults. Lastly, CD16/CD56 are the markers for natural killer cells (actually part of the innate rather than the adaptive immune system) and can range between 70 and 480 cells/microliter.

For PI, different types of isolated cellular immunodeficiency conditions, as well as combined humoral and cellular immunodeficiency syndromes, can present with decreased levels of various immune cell types. In addition, various secondary immunodeficiency conditions can present with low levels of immune cells such as infectious causes, medication-induced causes (i.e., being on immunosuppressive medications), increased loss due to enteropathy (chronic loss from the gastrointestinal tract) or decreased production due to bone marrow abnormalities.

In addition to assessing the quantity of adaptive immune cells, there are assays that will evaluate the quality and function of these cells. These are called the lymphocyte proliferation assays. These tests are not widely available (generally only offered in major academic tertiary referral centers). Both the B and T cells are extracted from the patient and are then exposed to different types of stimulants to determine whether the cells would grow and multiply normally. These tests are not easy to perform and rely on the comparison to the response of normal cells from healthy volunteers. The stimulants that are used can be non-specific, assessing whether the overall machinery of the B and T cells is functional, or they can be specific and examine whether the B and T cells can adequately respond to a particular foreign infectious agent. Reference ranges will depend on the method employed for the assays and can differ between laboratories. Furthermore, the ranges are also set by comparison to normal healthy volunteers. The importance of these assays is that they help the physician understand whether the cells of the adaptive immune system are functionally adequate regardless of the total quantity present.

### Demystifying the Diagnostic Process

There are many other laboratory tests in the field of clinical immunology assessing the potential of PI. The labs discussed in this article are most commonly performed for the evaluation of the most common types of PI. Like all laboratory evaluations, the results must not be interpreted in a vacuum and should always be coupled with the clinical presentation to arrive at the right diagnosis. PI labs are often difficult to interpret for both patients and providers. Hopefully, this article can help shed some light on the understanding of these tests and demystify the complexity of the diagnostic process. ■

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