The Importance of Blood Tests and Immunological Analyzes in Laboratory Medicine

By

Ibrahim Mohammed Ibrahim Alwadani*

***Technician Laboratory, East Jeddah hospital / JEDDAH**

Amal Ali Owaidh Alrefaei**

****Technician Laboratory , East Jeddah hospital / JEDDAH**

Abdulrhman Omar Ahmad Balubaid***

*****Technician Laboratory , East Jeddah hospital / JEDDAH**

Mohammed Ali Mohammed Alshanbari****

******Technician Laboratory , East Jeddah hospital / JEDDAH**

Assem Awad Ali Alghamdi*****

*******Technician Laboratory , East Jeddah hospital / JEDDAH**

Ahmad ataiq Ahmad Ataiq ALZAHRANI******

********Technician Laboratory , East Jeddah hospital / JEDDAH**

OMAR AHMED AGILI ALSHIKHI*******

*********Technician Laboratory , East Jeddah hospital / JEDDAH**

Alaa Abdullah Saeed Alghamdi********

**********Technician Laboratory , East Jeddah hospital / JEDDAH**

Samir Abdulla Ayed Alshamrani *********

***********Technician Laboratory , East Jeddah hospital / JEDDAH**

Mohammed Bander Khalif Alenazi **********

************Technician Laboratory , East Jeddah hospital / JEDDAH**

***Abstract*:**

The current study aimed to investigate the importance of blood tests and immunological tests in laboratory medicine. Autoimmune disease tests measure the amount of certain antibodies in the blood. Your body makes antibodies to attack and destroy substances such as bacteria and viruses. But in autoimmune diseases, antibodies attack and destroy your body's own tissues. This can lead to diseases such as rheumatoid arthritis, scleroderma, and lupus. These health problems affect connective tissues, such as the skin, joints, blood vessels, and other tissues.

Keywords: Blood Tests - Immunological Analyzes - Laboratory Medicine**.**

INTRODUCTION

Blood tests can be used to help a doctor identify a variety of health conditions, including infections, anemia, high cholesterol, vitamin deficiencies, organ failure, HIV, cancer, diabetes, and more.

Doctors use blood tests to analyze the behavior of substances like proteins, cells, or chemicals in your blood.

This can give them a picture of your overall health and help them diagnose different diseases, monitor chronic conditions, assess your organ function, and determine your immune system strength.

Regular blood testing is one of the most important ways to keep track of your overall physical well-being. We've partnered with Life force to bring you this overview article on blood tests.

THE IMPORTANCE OF BLOOD TESTS:

Before you even meet with your doctor for your annual physical or to discuss a medical concern you have, your doctor might have access to crucial information from your blood test. About 50 percent of the information in the average medical chart comes from laboratory data.

Diagnostic test results, including blood tests, inform approximately 70 percent of medical decisions.[1]

While a blood test is simple for you—with the exception of feeling "a little pinch"—the actual diagnostic process behind the scenes is quite complex, requiring specialized equipment and technicians. Even before your blood is tested, it needs to be properly prepared for the analyzer. It might be spun very fast to separate the blood cells from the fluid portion of the blood, creating a serum or plasma sample. Then the blood analyzer device counts and identifies the shape and size of blood cells, or measures chemical reactions to detect concentrations of certain molecules in blood. Finally, the results are verified by a trained lab professional and returned to your doctor.

"Many patients consider having a blood test to be a simple procedure, but don't understand what takes place behind the scenes between when the blood is drawn and when the doctor makes a diagnosis," said Dr. David Spindell, internist and divisional vice president of Medical Affairs, Diagnostic Products, Abbott. "Laboratory blood tests are a vital part of the diagnostic process, helping physicians make the correct diagnosis and determine the appropriate course of treatment."

Developing cutting-edge blood diagnostic tests and instruments is what Abbott is all about, enabling doctors to provide the best possible care more quickly and accurately.

- There are more than 100 types of blood tests available.
- The liquid portion of blood, plasma, constitutes 50-55% of the total blood volume.
- Some of today's automated diagnostic blood systems can process about 3,600 tests per hour.

Blood Test:

A blood test is a lab analysis of things that may be found in your blood. You may have blood tests to keep track of how well you are managing a condition, such as diabetes or high cholesterol. You may also have them for routine checkups or when you are ill.

Blood tests are very common. They are ordered by healthcare providers to:

- Find out how well organs, such as your kidneys, liver, heart, or thyroid are working
- Help diagnose diseases, such as cancer, diabetes, heart disease, and HIV/AIDS
- Find out if your medicine is working to make you better
- Diagnose bleeding or clotting disorders
- Find out if your immune system is having problems fighting infections
- Diagnose anemia, such as iron-deficiency anemia, pernicious anemia, aplastic anemia, or hemolytic anemia
- Find variations in hemoglobin, such as hemoglobin S, C, or E, which are common in people of African, Mediterranean, or Southeast Asian background
- Monitor chronic health conditions and diseases
- Find health problems in their early stages

You have the right to know why a blood test has been ordered and how much it will cost. Ask your healthcare provider if you are not sure why they want you to have the test.

Types of blood tests:

These are common blood tests:

- Complete blood count, also called a CBC
- Blood chemistry tests
- Blood enzyme tests
- Blood tests for heart disease risk

Blood tests can give your healthcare provider a lot of information. They can see if certain elements in your blood are in a normal range. But in many cases, blood tests are only part of the information your healthcare provider needs to make a diagnosis of a health condition. You might need to have some other types of tests as well.

Preparing for a blood test:

For most kinds of blood tests, you don't need to prepare. These tests are to see what your blood is like under normal conditions.

For some blood tests, you will have to fast for a certain amount of time before the blood test. The fasting time may vary depending on the test. These tests are often scheduled for early in the morning.

Your healthcare provider will let you know if you need to fast before a blood test.

The procedure:

To test your blood, a technician called a phlebotomist will use a needle to take a sample of blood. Tell the technician if the sight of needles makes you nervous. They can help you feel more at ease. You can also look away during the procedure, and bring a family member or friend to help distract you.

In most cases, the sample is taken from a vein in your arm. You will be seated or lying down. You may be asked to make a fist. The technician will tie a rubber band around your arm. Once they see a vein, the technician will clean the area and then insert the needle. You might feel a small prick or stinging sensation. Once the technician has drawn enough blood, they will take the needle out and put an adhesive bandage over the site. You may be asked to press firmly on the site to stop any bleeding.

10 important blood tests

Let's take a closer look at some common blood tests.

1. Complete blood count

A routine complete blood count (CBC) checks for levels of 10 different components of every major cell in your blood: white blood cells, red blood cells, and platelets.

Important components this test measures include red blood cell count, hemoglobin, and hematocrit.

Table 1

Component	Normal range
red blood cells (cells responsible for carrying oxygen throughout the body)	**male:** 4.5–6.1 × 10^6/ microleter (μL); **female:** 4.0–5.4 × 10^6/μL
white blood cells (immune system cells in the blood)	**male:** 4.0–10.8 × 10^3/μL **female:** 4.0–10.8 × 10^3/μL
platelets (the substances that control the clotting of the blood)	**male:** 150–400 × 10^3/μL **female:** 150–400 × 10^3/μL
hemoglobin (protein within the red blood cells that carries oxygen to organs and tissues and carbon dioxide back to the lungs)	**male:** 13.0–17.0 grams/deciliter (g/dL); **female:** 12.0–16.0 g/dL
hematocrit (percentage of blood made of red blood cells)	**male:** 40–52%; **female:** 37–47%

All About Blood Tests:

Blood tests can be used to help a doctor identify a variety of health conditions, including infections, anemia, high cholesterol, vitamin deficiencies, organ failure, HIV, cancer, diabetes, and more.

Doctors use blood tests to analyze the behavior of substances like proteins, cells, or chemicals in your blood.

This can give them a picture of your overall health and help them diagnose different diseases, monitor chronic conditions, assess your organ function, and determine your immune system strength.

Regular blood testing is one of the most important ways to keep track of your overall physical well-being. We've partnered with Lifeforce to bring you this overview article on blood tests.

Blood tests can help your doctor determine how different organs in your body are working. Examples of organs whose malfunctions can be visible in a blood test includeTrusted Source your heart, thyroid, liver, or kidneys.

Your doctor can also use blood tests to search for markers of diseases and health conditions such as:

- diabetes
- HIV
- anemia
- cancer
- coronary heart disease

Even if a person does not have heart disease, a blood test can show whether they may be at risk of developing the condition.

Other blood tests can indicate whether the medications you're taking are working properly or assess how well your blood is clotting.

When do you need a blood test?

You would typically undergo a blood test in the following cases:

- **During your annual physical exam:** Your doctor may order a general blood test such as the complete blood count (CBC).
- **You're at risk of a health condition**: You may need to undergo a specific blood test if you have a higher chance of developing a specific disease or condition or if you have a known genetic mutation that can cause a condition.
- **You have a known condition:** Sometimes,you already have a diagnosis, but your doctor needs to know how your condition is progressing or to evaluate your treatment.
- **You're having symptoms:** If you are experiencing symptoms, your doctor may need to run a blood test to confirm a suspected diagnosis or to see if you need more specialized testing.

- **You're pregnant:** During pregnancy, your doctor will do a CBC and test your blood type.
- **Before surgery:** You may need to do a blood test if your surgeon wants to check for anything that might put you more at risk during the procedure, such as excessive bleeding, for example.
- **You want to optimize your health**: Knowing the levels of various blood components, such as HDL and LDL cholesterol, can allow you to tweak your diet or fitness plan to maximize healthy habits.

Regarding routine blood tests, recommendations call for, at minimum, a lipid test starting at age 20 and every 5 years after that for people with a low risk of heart disease.

For people at a higher risk of heart disease, more frequent lipid testing may be necessary.

In addition, you should get a blood glucose test if you are 40-70 years old and overweight or have obesity.

People over age 45Trusted Source should start to be screened for colorectal cancer regularly. You may do a fecal occult blood test or a colonoscopy. Your doctor will tell you the appropriate testing for you.

1. A blood test can evaluate how well your organs are working – such as the kidneys and liver

A blood test is perhaps the most accessible preventive tool that identifies how well your organs are and how well they're functioning. A diagnosis for almost all organ-related issues starts with a blood test.

For instance, a simple potassium count through a blood test can assess the health of your kidneys. In the event, if your potassium count reaches a dangerous level, you'll be diagnosed with kidney disease followed by its treatment.

2. A blood test can tell what causes fatigue and shortness of breath

If you're often exhausted without any reason, it could be a sign that something is wrong. Fatigue is common among people with low iron or people with risks for heart disease.

To find out the exact cause of your fatigue, a FBC (Full blood count) test can count the number of blood cells in the blood. For instance, your clinician will count the number of red blood cells and hemoglobin in your blood for diagnosing anaemia (lower iron levels).

3. A blood test can explain your unexpected weight loss or weight gain

An unexplained weight loss or weight gain is one of the first few symptoms of a thyroid disorder. While weight loss is due to hyperthyroidism, weight gain occurs when your blood has lower thyroid hormone levels, causing hypothyroidism.

Through a blood test (thyroid test), your GP would be interested in measuring the amount of different hormones, including thyroid-stimulating hormone (TSH) and T4 hormones. The blood test can also measure the presence of antibodies to diagnose autoimmune thyroid disorders.

4. It can tell what nutrients you need more

Whether you're suffering from concurrent headaches, allergic to food, or confused about gluten-free food, a nutritional evaluation of your blood can provide crucial insights into your nutrient deficiency.

A nutritional test evaluates the levels of various macro and micronutrients such as protein (amino acids), fats, carbs, minerals, vitamins, and antioxidants.

5. Sexual health check through a blood test

Blood tests are used to diagnose many sexually transmitted infections such as HIV, genital herpes, and syphilis. If you're sexually active, it's essential to keep an eye on your sexual health.

If you find your nearest sexual health clinic in Queensland, click here.

6. A blood test can indicate diabetes

Your GP can diagnose diabetes, prediabetes, and gestational diabetes with the help of a blood test. Although you can buy testing equipment over the counter, using a blood glucose meter is not ideal for self-diagnosis.

A blood test allows your GP to find diabetes sooner and advice on its prevention and management.

7. A blood test can keep track of your health progress

Once you're diagnosed with a disease, for example, iron deficiency, a timely blood test can determine how well you're progressing in replenishing your iron levels to their healthy levels.

8. When a physical test fails, a blood test can do wonders

GPs may be unable to narrow down on your symptoms from just a physical examination. A simple physical examination can even fail to detect a condition leading to severe problems. In this case, a blood test will make it possible to close those gaps and find out exactly what's going on.

9. It can help find out whether you have risks for heart disease

Whether it's your high cholesterol level or other substances in your blood, a blood test can tell you a lot about your heart health.

For instance, a blood test can measure high levels of "bad" cholesterol, one of the primary risk factors for heart disease.

It can also measure the amount of fats in your blood, also called the lipid test.

10. It can assess how well your blood is clotting

A blood clot usually forms in the legs, termed thrombosis, but it can break loose and reach the arteries in the lungs causing life-threatening pulmonary embolism.

A blood test can detect the presence of these clots in the lungs, potentially saving your life.

The important blood tests:

Let's take a closer look at some common blood tests.

1. Complete blood count:

A routine complete blood count (CBC) checks for levels of 10 different components of every major cell in your blood: white blood cells, red blood cells, and platelets.

Important components this test measures include red blood cell count, hemoglobin, and hematocrit.

Here's the typical Trusted Source range of results, although every laboratory may have its own range that varies slightly:

Table 2

Component	Normal range
red blood cells (cells responsible for carrying oxygen throughout the body)	**male:** 4.5–6.1 × 10^6/microleter (μL); **female:** 4.0–5.4 × 10^6/μL
white blood cells (immune system cells in the blood)	**male:** 4.0–10.8 × 10^3/μL **female:** 4.0–10.8 × 10^3/μL
platelets (the substances that control the clotting of the blood)	**male:** 150–400 × 10^3/μL
	female: 150–400 × 10^3/μL
hemoglobin (protein within the red blood cells that carries oxygen to organs and tissues and carbon dioxide back to the lungs)	**male:** 13.0–17.0 grams/deciliter (g/dL); **female:** 12.0–16.0 g/dL
hematocrit (percentage of blood made of red blood cells)	**male:** 40–52%; **female:** 37–47%

The indicates:

Abnormal levels of these components may indicate :Trusted Source:

- Nutritional deficiencies, such as vitamin B6 or B12
- Anemia (iron deficiency).
- clotting problems.
- Blood cancer.
- Infection.
- Immune system disorders.

2. Basic metabolic panel:

A basic metabolic panel (BMP) usually checks for levels of eight compounds in the blood:

- calcium
- glucose
- sodium
- potassium
- bicarbonate
- chloride
- blood urea nitrogen (BUN)
- creatinine

This test may require you to fast for at least 8 hours before your blood is drawn, depending on the instructions of your doctor and what the test is measuring.

The indicates:

Abnormal results may indicate:

- kidney disease
- diabetes
- electrolyte imbalances

Your doctor will perform follow-up tests to confirm a diagnosis.

The Lifeforce Diagnostic is an at-home blood test designed to gather data on 40+ biomarkers that impact your health and longevity, including your metabolic condition, hormone health, and key risk factors for disease. Your diagnostic includes an at-home blood draw from an experienced phlebotomist, a telehealth consultation with a Lifeforce clinician, and a personalized plan that consists of expert insights, lifestyle improvements, nutraceuticals, and hormone and peptide therapies.

3. Comprehensive metabolic panel:

A comprehensive metabolic panel (CMP) includes all the measurements of a BMP as well as additional proteins and substances related to liver function, such as:

- albumin.
- total protein.
- alkaline phosphatase (ALP), an enzyme mostly found in the bones and liver that's involved in several bodily processes.
- alanine aminotransferase (ALT), an enzyme found in the liver.
- aspartate aminotransferase (AST), an enzyme found in the liver and other tissues within the body.
- bilirubin, which is waste resulting from the breakdown .of red blood cells that the liver filters out.

The indicates:

The same conclusions can be drawn from a CMP as from a BMP for the same substances that a BMP covers. Other abnormal levels can also indicate underlying conditions, such as:

Table 3

	High levels	**Low levels**
ALP	• bile duct blockage • cirrhosis • gallbladder inflammation • gallstones • hepatitis • mononucleosis • Paget's disease	• bone metabolism disorders • heart surgery • malnourishment • zinc deficiency
ALT	• cirrhosis • hepatitis • liver cancer • liver damage	considered normal
AST	• cirrhosis • heart conditions • hepatitis • mononucleosis • (mono)pancreatitis	considered normal
bilirubin	• abnormal red blood cell destruction (hemolysis) • adverse medication reactions • bile duct blockage • Gilbert's syndrome • hepatitis	not a concern

4. Lipid panel:

This test checks levels of two typesTrusted Source of cholesterol:

- high-density lipoprotein (HDL), or "good" cholesterol
- low-density lipoprotein (LDL), or "bad" cholesterol

HDL is "good" because it removes harmful substances from your blood and helps the liver break them down into waste. LDL is "bad" because it can cause plaque to develop in your arteries, increasing your risk of heart disease.

You may need to fast for at least 8 hours before this test.

For HDL cholesterol, 60 milligrams per deciliter (mg/dL) or above is considered to be heart-healthy, while under 40 mg/dL is a major risk factor for heart disease.

For LDL cholesterol, 100 mg/dL or below is optimal for good health, while 160 mg/dL or over is dangerously high.

Normal levels can also vary by age.

5. Thyroid panel:

A thyroid panel, or thyroid function test, checks how well your thyroid is producing and reacting to certain hormones, such as:

- **Triiodothyronine (T3)**: Along with T4, this regulates your heart rate and body temperature.
- **Thyroxine (T4)**: Along with T3, this regulates your metabolism and how you grow.
- **Thyroid-stimulating hormone (TSH)**: This helps regulate the levels of hormones your thyroid releases.

Your thyroid is a tiny gland in your neck. It helps regulate bodily functions like your mood, energy level, and overall metabolism.

Normal results:

- **T3:** 80–180 nanograms per deciliter of blood (ng/dL)
- **T4:** 0.8–1.8 ng/dL in adults.

- **TSH:** 0.5–4 milli-international units per liter of blood (mIU/L)

The indicates:

Abnormal levels of these hormones can indicate numerous conditions, such as:

- low protein levels
- thyroid growth disorders
- abnormal levels of testosterone or estrogen

6. Cardiac biomarkers:

Enzymes are proteins that help your body accomplish certain chemical processes, such as breaking down food and clotting blood. They're used throughout your body for many vital functions.

The normal ranges for the enzymes listed above:

- **hs-cTn:** <1 ng/mL
- **BNP:** <100 picograms per milliliter (pg/mL)
- **NT-proBNP:** ≤300 pg/mL
- **CK:** 30–200 units per liter (U/L)
- **CK-MB:** 0–12 IU/L

The indicates:

Abnormal enzyme levels can indicate many conditions.

Common enzymes tested include:

- **High-sensitivity cardiac troponin (hs-cTn):**This is a heart enzyme that can leak into your blood and result in heart injury.
- **B-type natriuretic peptide (BNP) and N-terminal pro b-type natriuretic peptide (NT-proBNP)**: These substances are created in the heart. High levels may be an indication of congestive heart failure.
- **Creatine kinase (CK)**: This enzyme is primarily located in the brain, heart, and skeletal muscle. When muscle damage happens, CK seeps into the blood in growing amounts.
- **Creatine kinase-MB (CK-MB)**: These enzymes are found in your heart. They often increase in your blood after a heart attack or other heart injury.

7. Sexually transmitted infection tests:

Many sexually transmitted infections (STIs) can be diagnosed using a blood sample. These tests are often combined with urine samples or swabs of infected tissue for more accurate diagnoses.

The following STIs can be diagnosed with blood tests:

- herpes
- HIV
- syphilis
- hepatitis C

Blood tests aren't always accurate right after contracting an infection. For an HIV infection, for example, you may need to wait at least a month before a blood test can detect the virus.

8. Coagulation panel:

Coagulation tests measure how well your blood clots and how long it takes for your blood to clot. Examples include the prothrombin time (PT) test and fibrinogen activity test.

Clotting is a crucial process that helps you stop bleeding after a cut or wound. However, a clot in a vein or artery can be deadly since it can block blood flow to your brain, heart, or lungs. This can cause a heart attack or stroke.

Coagulation test results vary based on your health and any underlying conditions that may affect clotting.

The indicates:

Results from this test can be used to diagnose:

- excessive bleeding (hemophilia)
- thrombosis
- liver conditions
- vitamin K deficiency

9. DHEA-sulfate serum test:

The dehydroepiandrosterone (DHEA) hormone comes from your adrenal glands. This test measures whether it's too high or too low.

In men, DHEA helps develop traits like body hair growth, so low levels are considered abnormal. In females, high levels can cause typically male traits, like excess body hair, to develop, so low levels are normal.

Low levels may be caused by:

- Addison's disease.
- adrenal dysfunction.
- hypopituitarism.
- premature puberty in females.
- use of steroid medications.

The indicates:

High levels in males or females can result from:

- congenital adrenal hyperplasia.
- benign or malignant tumor on the adrenal gland.
- polycystic ovary syndrome (PCOS).
- ovarian tumor.

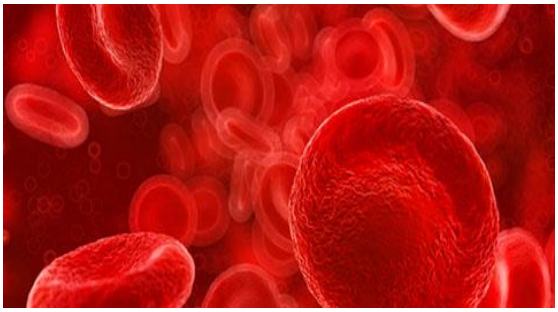
10. C-reactive protein test:

C-reactive protein (CRP) is made by your liver when tissues in your body are inflamed.

The higherTrusted Source the level, the higher the risk of heart disease:

- **<0.3 mg/dL:** normal
- **0.3 to 1.0 mg/dL:** minor elevation can be associated with a person's sex, body mass index (BMI), or with conditions like depression or insomnia
- **1.0 to 10.0 mg/dL:** moderate elevation usually caused by systemic inflammation, such as from an autoimmune disease, bronchitis, heart attack, or cancer
- **>10.0 mg/dL:** marked elevation typically caused by a serious bacterial or viral infection, major trauma, or systemic vasculitis
- **>50.0 mg/dL:** severe elevation usually caused by an acute bacterial infection.

Figure1



The indicates:

High CRP levels indicate inflammation from a variety of causes, including:

- bacterial or viral infection
- autoimmune diseases, such as Lupus or rheumatoid arthritis
- inflammation related to diabetes
- inflammation related to physical trauma or from habits like smoking
- cancer.

Types of blood tests:

Blood tests are very common. They help doctors check for certain diseases and conditions. They also help check the function of your organs and show how well treatments are working.

Figure2

Complete blood count (CBC):

The complete blood count (CBC) is one of the most common blood tests. It is often done as part of a routine checkup. This test measures many different parts of your blood, including red blood cells, white blood cells, and platelets.

- **Red blood cell levels** that are higher or lower than normal could be a sign of dehydration, anemia, or bleeding. Red blood cells carry oxygen from your lungs to the rest of your body.
- **White blood cell levels** that are higher or lower than normal could be a sign of infection, blood cancer, or an immune system disorder. White blood cells are part of your immune system, which fights infections and diseases.
- **Platelet levels** that are higher or lower than normal may be a sign of a clotting disorder or a bleeding disorder. Platelets are blood cell fragments that help your blood clot. They stick together to seal cuts or breaks on blood vessel walls and stop bleeding.
- **Hemoglobin levels** that are lower than normal may be a sign of anemia, sickle cell disease, or thalassemia. Hemoglobin is an iron-rich protein in red blood cells that carries oxygen.
- **Hematocrit levels** that are too high might mean you're dehydrated. Low hematocrit levels may be a sign of

anemia. Hematocrit is a measure of how much space red blood cells take up in your blood.

- **Mean corpuscular volume (MCV) levels** that are lower than normal may be a sign of anemia or thalassemia. MCV is a measure of the average size of your red blood cells.

The table below shows some normal adult ranges for different parts of the CBC test. Some of the normal ranges differ between men and women. Other factors, such as age, high altitude, and race, also may affect normal ranges.

Your healthcare provider should discuss your results with you. They will advise you further if your results are outside the normal range for your group.

Table 4

Test	Normal Range Results*
Red blood cell	Adult Men: 5 to 6 million cells/mcL Adult Women: 4 to 5 million cells/mcL
White blood cell	4,500 to 10,000 cells/mcL
Platelets	140,000 to 450,000 cells/mcL
Hemoglobin (varies with altitude)	Adult Men: 14 to 17 gm/dL Adult Women:12 to 15 gm/dL
Hematocrit (varies with altitude)	Adult Men: 41% to 50 % Adult Women: 36% to 44%
Mean corpuscular volume	0 to 95 femtoliter†

*** Cells/mcL = cells per microliter; gm/dL = grams per deciliter.**
† A femtoliter is a measure of volume.

1. A blood test can evaluate how well your organs are working – such as the kidneys and liver:

A blood test is perhaps the most accessible preventive tool that identifies how well your organs are and how well they're functioning. A diagnosis for almost all organ-related issues starts with a blood test.

For instance, a simple potassium count through a blood test can assess the health of your kidneys. In the event, if your potassium count reaches a dangerous level, you'll be diagnosed with kidney disease followed by its treatment.

2. A blood test can tell what causes fatigue and shortness of breath:

If you're often exhausted without any reason, it could be a sign that something is wrong. Fatigue is common among people with low iron or people with risks for heart disease.

To find out the exact cause of your fatigue, a FBC (Full blood count) test can count the number of blood cells in the blood. For instance, your clinician will count the number of red blood cells and hemoglobin in your blood for diagnosing anaemia (lower iron levels).

3. A blood test can explain your unexpected weight loss or weight gain:

An unexplained weight loss or weight gain is one of the first few symptoms of a thyroid disorder. While weight loss is due to hyperthyroidism, weight gain occurs when your blood has lower thyroid hormone levels, causing hypothyroidism.

Through a blood test (thyroid test), your GP would be interested in measuring the amount of different hormones, including thyroid-stimulating hormone (TSH) and T4 hormones. The blood test can also measure the presence of antibodies to diagnose autoimmune thyroid disorders.

4. It can tell what nutrients you need more :

Whether you're suffering from concurrent headaches, allergic to food, or confused about gluten-free food, a nutritional evaluation of your blood can provide crucial insights into your nutrient deficiency.

A nutritional test evaluates the levels of various macro and micronutrients such as protein (amino acids), fats, carbs, minerals, vitamins, and antioxidants.

5. Sexual health check through a blood test :

Blood tests are used to diagnose many sexually transmitted infections such as HIV, genital herpes, and syphilis. If you're sexually active, it's essential to keep an eye on your sexual health.

6. A blood test can indicate diabetes:

Your GP can diagnose diabetes, prediabetes, and gestational diabetes with the help of a blood test. Although you can buy testing equipment over the counter, using a blood glucose meter is not ideal for self-diagnosis.

A blood test allows your GP to find diabetes sooner and advice on its prevention and management.

7. A blood test can keep track of your health progress :

Once you're diagnosed with a disease, for example, iron deficiency, a timely blood test can determine how well you're progressing in replenishing your iron levels to their healthy levels.

8. When a physical test fails, a blood test can do wonders :

GPs may be unable to narrow down on your symptoms from just a physical examination. A simple physical examination can even fail to detect a condition leading to severe problems. In this case, a blood test will make it possible to close those gaps and find out exactly what's going on.

9. It can help find out whether you have risks for heart disease:

Whether it's your high cholesterol level or other substances in your blood, a blood test can tell you a lot about your heart health.

For instance, a blood test can measure high levels of "bad" cholesterol, one of the primary risk factors for heart disease.

It can also measure the amount of fats in your blood, also called the lipid test.

10. It can assess how well your blood is clotting:

A blood clot usually forms in the legs, termed thrombosis, but it can break loose and reach the arteries in the lungs causing life-threatening pulmonary embolism.

A blood test can detect the presence of these clots in the lungs, potentially saving your life.

IMMUNOLOGICAL ANALYZES IN LABORATORY MEDICINE:

History

In 1916, the first formal Department of Immunology in the United States of America was founded at Johns Hopkins University. This rich heritage of Immunology was initiated by Dr. William Henry Welch who launched his vision of an Immunology department with an emphasis on research and teaching that soon spread to a variety of diverse programs at Johns Hopkins. Today, these programs have expanded into centers focusing on basic immunology, translational research and direct applications to patient management.

The Immunology Lab

The Immunology Laboratory is the clinical component of the Immunology Division. The laboratory performs and offers expert interpretation on a broad array of laboratory tests and comprehensive consultation in clinical and diagnostic immunology. In addition, the laboratory is involved in research and the development of diagnostic tests for a wide range of immune-based disorders. Approximately 300,000 tests are performed each year for the evaluation of autoantibodies, protein abnormalities and antibody responses to selected microbial agents. The Laboratory is managed by Annie Kuh and a staff of 16 highly skilled medical laboratory professionals.

In 2016, Dr. Patrizio (Mario) Caturegli became the director of the Immunology Laboratory taking over from Dr. Barbara Detrick who led the laboratory from 1999-2016. Today, the Immunology Laboratory continues to grow and provide a wide variety of services in immunologic testing. The evaluation of monoclonal proteins is a basic component of the laboratory analysis for multiple myeloma and other plasma cell dyscrasias. Consultation is provided by an expert group of faculty.

Another component of the laboratory is autoimmune disease testing. This section monitors autoantibodies that are generated in a variety of diseases such as, systemic lupus erythematosus, Sjögren's Syndrome, rheumatoid arthritis, myositis and celiac disease. The laboratory is also dedicated to infectious disease serology. A broad spectrum of infectious disease serology is offered, ranging from Lyme disease to syphilis. A change was initiated in syphilis testing with the advent of the reverse sequence algorithm, approved by the CDC for syphilis testing. Initial screening is performed by chemiluminenscence (CIA), followed by the traditional RPR test, and confirmed with the Treponemal pallidum particle agglutination assay.

In addition, the Immunology Laboratory is dedicated to the academic development of fellows, residents, medical students, graduate students, and medical technologists. Pathology residents rotate for two months through all areas of the Immunology Laboratory.

Immunologic testing is an integral part of several areas related to immunology, embracing basic and applied research, clinical laboratory routine, epidemiological survey, blood bank control, and *in vitro* diagnostic industry (IVD) research, development, and production, just to mention a few. The complex network of the immune system, modeled by myriad soluble and surface molecules and multiple circulating and resident cells, reflects the great variety of "immunologic analytes" to be determined in the various immunologic tests addressing the diverse areas in which immunology plays a relevant role. These encompass a broad spectrum spanning several medical specialties, including allergic and autoimmune diseases, primary and secondary immunodeficiencies , infectious diseases, cancer, vaccination,

and epidemiology. Aside from immune-related diseases, immunoassays are also crucial tools in most areas of medicine, from endocrinology to toxicology, as exemplified by immunoassays for the determination of hormones, therapeutic drugs, serum proteins, vitamins, and tumor biomarkers, among others.

Standardization and quality assessment are crucial for any laboratory analysis so that results obtained in different laboratories and different parts of the world share a minimum degree of coherence. Each analyte to be determined has peculiar characteristics that affect the respective laboratory assay and, consequently, affect the way these assays need to be standardized and controlled. The myriad analytes addressed in immunologic testing display multiple peculiarities, rendering standardization and quality assessment in immunology a complex and multifaceted field. Some molecules do not show relevant polymorphism, such as C-reactive protein, soluble IL-2 receptor, and complement factor C1q. In contrast, some other targets of immunology testing represent the most polymorphic elements in biology, such as the major histocompatibility complex genes and ensuing proteins. Cytokines and several complement components are extremely labile, requiring specific pre-analytical handling, whereas immunoglobulins are rather stable at room temperature for several hours. Samples for cryoglobulin determination must be handled at 37°C during the entire pre-analytical stage because these peculiar immunoglobulins may precipitate, becoming trapped in the blot clot, which would yield false negative results. These are just a few examples of the particularities of immunologic analytes that influence the standardization of immunologic assays.

A substantial branch of immunology testing refers to the determination of antibodies specific to a certain target, be it a microorganism, an autoantigen, an allergen, an alloantigen, or a toxin. In fact, these assays are set to determine the humoral immune response to a given antigen and this is not represented by a monoclonal antibody, but rather by a polyclonal collection of antibodies that share that antigen as their target. Considering the polymorphism of the immunoglobulin genes and the random dynamics of the development of the antibody response, it is obvious that each individual forms a distinctive collection of antibodies against each antigen. The mosaic of antibodies in each individual is analogous to a "fingerprint" characterized by different proportions of antibodies with different isotypes, targeted epitopes, avidities, and Fc post-translational modifications (glycosylation, acetylation, etc.), all these being balanced at different serum concentrations. In a sense, the panel of anti-X antibodies in individual A will be necessarily different from the panel of anti-X antibodies in individual B. Under this perspective, it is easy to realize that any given immunoassay to determine anti-X antibodies will perform differently for different individuals, and different immunoassays for anti-X antibodies can yield different results in the same sample. In fact, in contrast to simple analytes (all molecules are the same across individuals) such as glucose and C-reactive protein, antibodies are complex analytes (each individual has its own array of molecules) that represent the functional response of the humoral immune system against a given antigen. This scenario brings a considerable challenge for the IVD industry in developing products that perform appropriately for a relevant part of the population of interest. However, the biggest challenge is the standardization and harmonization of proprietary immunoassays of dozens of IVD industries originated in different parts of the world, calibrated, and validated using samples from patients from diverse ethnic and environmental backgrounds.

In order to handle the challenge of standardization in immunology testing, the International Union of Immunology Societies (IUIS) has established a committee dedicated to Quality Assessment and Standardization (QAS) in Immunology. The QAS Committee operates for over four decades by means of specific subcommittees, namely, the Allergen Standardization Subcommittee (1), the Autoantibodies in Rheumatic and Related Diseases Subcommittee (2), the Complement Subcommittee (3), the Leukocytes Subcommittee (4, 5, www.hcdm.org), and the Big Data in Immunology subcommittee (https://iuis.org/committees/qas/big-data-for-immunology-sub-committee/). Each of these subcommittees coordinates various actions aiming to promote quality assessment and standardization in their respective field. These actions include the preparation and distribution of reference materials (standards), the establishment of guidelines and policies, and educational activities. The Research Topic *Contemporary challenges in immunologic testing in clinical and research laboratories* is a recent initiative from the QAS Committee and addresses several aspects of interest in the area.

Serological immunoassays for the diagnosis of infectious diseases have been a major priority in research, IVD industry, and clinical laboratories. Although this activity has been flourishing for decades, the recent Severe Acute Respiratory Disease Coronavirus 2 (SARS-CoV-2) pandemic has brought to spotlight the crucial role of serologic immunoassays in the management of infectious diseases. In the early days of the pandemic, robust and reliable serological immunoassays should be promptly developed to characterize the abundance, neutralization efficiency, and duration of antibodies associated with the humoral immune responses to SARS-CoV-2. In addition to the use of these tests for the management of individual patients, the accurate detection, measurement, and characterization of the anti-SARS-CoV-2 humoral response (i.e., temporal dynamics, isotype distribution, neutralization capacity) has been critical for vaccine development, establishment of guidelines for healthcare and at-risk workers, and monitoring reinfections with genetic variants of the virus. All these aspects were brilliantly covered in this Research Topic by Galipeau et al. who also address the benefits and limitations of the currently available commercial and laboratory-based serological assays, in

addition to the potential of cross-reactivity and possible immunological back boosting by seasonal coronaviruses.

The urgent need for a low-cost assay to diagnose dengue efficiently is addressed in the manuscript by Lai et al. This is especially relevant since no commercial dengue antigen tests able to differentiate viral serotypes are available. The authors have developed a multiplex lateral flow immunoassay (LFIA) that can identify mono- and co-infection of different serotypes of dengue viruses in mosquitoes. This new assay provides a simple tool for the rapid detection of dengue and is efficient for the differential diagnosis of fever patients in regions where medical resources are limited.

Another area of great contemporary interest is the field of immunobiological drugs embracing monoclonal antibodies and fusion proteins targeting key elements of the immune system with the aim of modulating and controlling inflammatory and autoimmune disorders. Initiating in the mid-1990s, this therapy modality has proven to be able to change the natural history of a host of chronic and disabling diseases such as rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, neuromyelitis optica, just to cite a few (6). A plethora of monoclonal antibodies and their respective molecular targets is currently part of the routine jargon of physicians and patients and the area is in frank expansion. Lately, several of the original monoclonal antibodies have been licensed to be produced as biosimilar drugs. In parallel, the concept of therapeutic drug monitoring has been established with the aim of achieving the most appropriate drug serum levels and optimizing the therapeutic results. This scenario clearly indicates an urgent need for harmonization and standardization of the original immunobiological drugs and their biosimilar correlates with respect to pharmacokinetics and bioactivity. One key element for standardization in the field is the establishment of International Standards (IS) for each monoclonal antibody. In this Research Topic, Wadhwa et al. originally present the first World Health Organization IS for adalimumab, a leading anti-TNF-α monoclonal antibody. This IS will have great utility in a wide range of applications, including the validation, calibration, and standardization of bioassays for measuring adalimumab and biosimilar effectivity, as well as immunoassays to determine adalimumab/biosimilar serum levels in therapeutic drug monitoring.

The screening for autoantibodies using the indirect immunofluorescence assay on HEp-2 cells (HEp-2 IFA) is widely used in the diagnostic investigation of patients suspected of systemic autoimmune diseases. The immunofluorescence pattern elicited by reactive samples is very useful because it provides indirect information on the probable antigenic targets of the autoantibodies in the sample. This topic has been largely developed by the International Consensus on ANA Patterns (ICAP) initiative (7, 8, www.anapatterns.org). In this Research Topic, Röber et al. present an international multicenter study establishing a novel HEp–2 IFA pattern strongly associated with autoantibodies to SS–A/Ro 60kDa, an autoantibody observed in patients with systemic lupus erythematosus and Sjögren's syndrome.

Dozens of competent IVD industries offer convenient kits with slides containing fixed HEp–2 cells and all the reagents necessary for the HEp–2 IFA procedure. It has been demonstrated that the HEp–2 IFA pattern produced by a given sample may vary according to the conditions used to cultivate and fix the cells (9). In this Research Topic, Silva et al. provide an extensive analysis of the HEp–2 IFA pattern observed in four high–ranked HEp–2 IFA kits using 900 samples from individuals with an array of clinical conditions. They found that non–reproducibility of the HEp–2 IFA pattern is rather prevalent and occurs more frequently in samples with weaker reactivity (lower titer) as well as in some specific patterns (e.g., nucleolar patterns).

In addition, HEp–2 IFA–reactive samples from healthy individuals tended to present non–reproducibility of results among HEp–2 IFA kits more often than samples from patients with systemic autoimmune diseases (Silva et al.). The non–reproducibility phenomenon demonstrated by Silva et al. should have an important impact on the clinical use of the HEp–2 IFA test and, therefore, international initiatives are needed to promote the harmonization of the properties and performance of HEp–2 IFA commercial kits.

Recent developments in modern complement analysis have been addressed by Frazer–Abel et al. Dysregulation and over–activation of the complement system are major causes of a variety of inflammatory and autoimmune diseases ranging from nephropathies, age–related macular degeneration (AMD), and systemic lupus erythematosus (SLE) to graft rejection, sepsis, and multi–organ failure. The clinical relevance of the complement system to immunologic diseases is reflected by the recent development of multiple drugs targeting complement with a broad spectrum of indications. The recognition of the role of complement in diverse diseases and the advent of complement therapeutics has increased the number of laboratories and suppliers entering the field. This has highlighted the need for reliable complement testing. The relatively rapid expansion in complement testing has presented challenges for a previously niche field. This is exemplified by the issue of cross–reactivity of complement–directed antibodies and by the challenges of the poor stability of many of the complement analytes, esp. of complement activation products. The complex nature of complement testing and increasing clinical demand has been met in the last decade by efforts to improve standardization among laboratories. Initiated by the *IUIS/ICS (International Complement Society) Committee for the Standardization and Quality Assessment in Complement Measurements*, 14 rounds of external quality assessment since 2010 resulted in improvements

in the consistency of testing across participating institutions while extending the global reach of the efforts to meanwhile more than 300 laboratories in 30 countries. Worldwide trends of assay availability, usage, and analytical performance are summarized based on the experience from recent years. Progress in complement analysis has been facilitated by the quality assessment and standardization efforts that now allow complement testing to provide a comprehensive insight into deficiencies and the activation state of the system. This in turn enables clinicians to better define disease severity, evolution, and response to therapy.

Dysregulation of the complement system also contributes to the pathogenesis of preeclampsia, which is mainly characterized by gestational hypertension, proteinuria, systemic endothelial cell activation, and inflammatory overreaction. In search for appropriate biomarkers, Liu et al. investigated the levels of adipsin, C3a, C5a, and soluble endoglin (sENG) before delivery to assess their role in preeclampsia. Then, a follow–up analysis was conducted to determine whether complement levels and sENG fluctuate with gestational age and whether plasma adipsin and related important circulating complement molecules can be used as an early–pregnancy predictor and potential diagnostic biomarkers of preeclampsia (Liu et al.).

They found that adipsin is likely a novel plasma biomarker to monitor the increased risk of preeclampsia in early pregnancy. Moreover, the increased plasma levels of adipsin, C5a, and sENG before delivery may be associated with preeclampsia.

Recurrent angioedema without urticaria (AE) in its hereditary (HAE) or acquired (AAE) form is commonly misdiagnosed due to restricted access and availability of appropriate laboratory tests. HAE with C1 inhibitor defect (HAE–C 1–INH) is associated with quantitative and/or functional deficiency of this multifunctional regulator. Although this bradykinin–mediated disease results mainly from a disturbance in the kallikrein–kinin system, traditionally complement evaluation has been used for diagnosis. Diagnosis is established by nephelometry, turbidimetry, or radial immunodiffusion for quantitative measurement of C1 inhibitor, and chromogenic assay or ELISA has been used for functional C1–INH analysis. However, as reviewed by Grumach et al. in this Research Topic, a large group of patients present with similar clinical manifestations to HAE but without C1–INH defect and normal C4 (HAE–nlC1–INH). Although a causative mutation cannot be found in a considerable number of patients with HAE–nlC1–INH, new variants in several genes have been associated recently with this form of the disease, such as angiopoietin 1 gene, plasminogen, kininogen, myoferlin, and heparan sulfate 3–O–sulfotransferase 6 genes. These new mutations not only imply novel mechanisms and systems involved in the pathogenesis of HAE but also open the possibility for new biomarkers and treatment targets.

The interesting paper by Kužílková et al. deals with the problem of a lack of reproducible identification of leukocyte subsets. The authors describe the development of a flow cytometric procedure for quantitative expression profiling of surface antigens on blood leukocyte subsets, which is standardized across multiple research laboratories. This workflow, bioinformatics pipeline, and optimized flow panels enable the mapping of the expression patterns of Human Leukocyte Differentiation Antigen (HLDA)–approved mAb clones to cluster of differentiation (CD) markers, benchmarking new antibody clones to established CD markers, and defining new CDs in future HLDA workshops.

The Opinion article by Di Rosa et al. discussed advances in the field of T cell proliferation analysis. It challenges the well–established idea that Ki–67 per se is an ideal marker of T cell proliferation. They propose the use of a new Ki–67/DNA dual staining, or TDS assay, which represents a more reliable approach by which human peripheral blood can be used to reflect the dynamics of human lymphocytes, rather than providing mere steady–state phenotypic snapshots.

The broad range of immunologic tests performed in clinical and research laboratories is in frank expansion and affects most areas of medicine. Quality assessment and standardization in immunology testing is a fundamental aspect that meets several challenges elicited by the peculiar characteristics of several of the immunologic analytes to be determined.

International organizations dedicated to promoting standardization and quality assessment in different areas of immunology testing contribute substantially to the progress in the area. The IVD industry provides a variety of commercial kits, contributing to the widespread availability of immunology testing in clinical and research laboratories in most parts of the world. However, the plethora of commercial kits available adds an exceptional challenge to the standardization of the tests. Although these commercial products are licensed by official regulatory agencies, there is no formal collaboration between these official agencies and the international quality assessment and standardization initiatives formed by specialists in each area. A tripartite collaboration involving the IVD industry, international specialists, and official regulatory agencies has the genuine potential to improve significantly the standardization and harmonization of immunology testing worldwide.

Immunological tests should be preceded by a thorough evaluation of anamnestic data and clinical examination results, as many symptoms may signalise potential immune-mediated diseases. Also standard laboratory tests, biochemical, haematological, cytological or histological analyses and visualisation (radiography) methods may be of a great value for an immune-mediated disease diagnosis. Accordingly, immunological tests should be only performed in the case of an adequate indication, with respect to their costs and cogency. Diagnostic criteria have been established for various immune-

mediated diseases, with laboratory analysis playing major or minor roles, according to their diagnostic value.

Laboratory Tests:

Hours: Monday - Friday, 7 am to 5 pm, Meyer B120 (*Limited Syphilis coverage on Saturdays and Sundays)*

Contact: Phone: 410.955.6570 (7am-4pm) | Fax: 410.614.7314

Resident: Phone: 410.955.2737 | Pager: 410.283.3406

Specimen Requirements :

- Cryoglobulin specimens require special handling. Samples are collected in red top tubes and must be kept at 37degrees Celsius from collection through transport to the laboratory.
- CSF samples should be collected in sterile screw-capped tubes.
- Serum samples should be collected in serum separator tubes.
- Urine samples should be collected in urine containers without preservatives or other additives.

Educational Programs:

Pathology Residents:

Clinical Pathology residents have a two-month rotation in the Immunology Laboratory. During this time, they will gain both a practical and theoretical knowledge of the performance and the interpretation of the various immunologic tests offered in this laboratory, which involves cases relating to monoclonal gammopathies, autoimmune conditions and the serological aspect of infectious disease states.

Allied Health Program:

Medical Technology and Medical Laboratory Technician students in these undergraduate programs become familiar with testing methodologies performed in the Immunology Laboratory. We currently host students from the University of Maryland, the University of Delaware, Andrews University, Stevenson University, Morgan State University, and Community College of Baltimore County.

Middle & High School Outreach:

A unique outgrowth of the Allied Health Program is the outreach to area middle and high schools. Staff from the Immunology laboratory, as well as other Johns Hopkins Hospital laboratories, participate in programs to promote interest in the sciences at Paul Laurence Dunbar Middle & Senior High Schools.

Specialized Rotations:

Other rotations and/or laboratory observations are specifically tailored to meet the needs of Fellows, medical and laboratory students and visiting scientists and guests from all over the globe.

Immunology Faculty and staff participate in the following additional education endeavors:

- Autoimmunity Day: a full day activity in which Johns Hopkins faculty and invited guests update their colleagues on their latest research findings.
- Immunology Teaching-two-week period of Immunology Lectures (an Immunology Section for second year medical students)
- Graduate Immunology - PhD graduate students in the Pathobiology program, which includes clinical laboratory rotations tailored to the students' interests
- Selected courses in Graduate Immunology in the Bloomberg School of Public Health

Immunological laboratory diagnostic methods can be classified from several aspects:

I. Based on a group of diseases that facilitate diagnosis

▪ Immunological profile tests for the detection of immunodeficiency

▪ Hypersensitivity tests

▪ Autoimmunity tests

II. Based on availability of the methods

▪ Methods performed in a surgery

▪ Methods included in haematological or biochemical analysis, and histological or visualisation methods that provide valuable information for immunological diagnosis

▪ A group of basic methods conducted in a specialised immunological laboratory

▪ Advanced immunological methods above all, used in clinical research

Whereas laboratory diagnosis of allergic and autoimmune diseases is based on serological examination and the tests are usually available as commercially produced kits, technically demanding methods are necessary for the detection of immunodeficiency or immunosuppression disorders.

Basic Laboratory Examinations:

Leukocyte count and differential leukocyte count, i.e., standard haematological parameters should be included in basic laboratory examinations, commonly available in any small animal practice. Cytology and histology of various samples obtained from biopsies are also of great value for immunological diagnosis. Preliminary methods selected for humoural immunity testing involve the assessment of total immunoglobulins using a simple precipitation method or serum electrophoresis. Among inflammatory parameters, the C-reactive protein test is available as a commercial kit in dogs however it is not commonly used, due to its cost versus diagnostic value.

Allergy Tests :

Practical veterinary surgeons have available in their consulting rooms the hypersensitivity skin tests for allergy diagnosis, including tests for the detection of a particular allergen. These tests produce quite reliable results especially in canine atopy. Allergens are commercially available and tests are usually used in practice for the detection of hypersensitivity type I. Nevertheless, hypersensitivity type IV detection is also relevant.

Nowadays, the diagnostic value of intradermal skin tests is comparable with that of serological detection of specific IgE antibodies against respective allergens using commercially available ELISA kits.

Detection of Autoantibodies

Detection of autoantibodies is an important diagnostic tool for diagnosis of autoimmune diseases. Despite the fact that their occurrence is not quite specific for a respective disease, it may considerably facilitate the diagnosis. Human immunological laboratories have available a wide range of commercial tests, whereas the offer in veterinary medicine is somewhat limited.

Analysis of circulating immune complexes by their non-specific precipitation in sera with polyethyleneglycol is an auxiliary method for the detection of hypersensitivity type III status. However, elevated concentration of circulating immune complexes is also usually detected during chronic infectious processes and due to this fact, the result of the analysis does not lead to a specific conclusion.

Antinuclear antibodies (ANA) are characteristic for systemic autoimmune diseases, above all: systemic lupus erythematosus (SLE). They are detected by indirect immunofluorescence, in sera. A significant level of antibodies (titre 80-100) and characteristic localisation (granular or homogenous fluorescence of the nucleus) is a precursor for obtaining a positive result in the test. The diagnostic value of the test is relatively high.

Rheumatoid factor (RF) is the antibody against Fc fragment of immunoglobulin, in dogs this is usually against IgG, the RF isotype being IgM, or IgA. Rheumatoid factor detection is performed by various tests: conventional Rose-Waaler test, or most recently by turbidimetry or ELISA methods. The diagnostic value of RF detection that should be characteristic of rheumatoid arthritis is low, because rheumatoid factor is also found in serum during other autoimmune diseases, chronic inflammatory responses and even in the serum of normal (especially older) animals.

Immune-mediated anaemia is characterised by the presence of autoantibodies and/or the C3 component of complement proteins on the surface of erythrocytes from a patient. **Direct antiglobulin (Coombs) test** is most convenient for their detection, as it reveals when autoantibodies or complement proteins are bound to the surface of a patient's erythrocytes. A positive reaction of agglutination signalises an immune-mediated cause of anaemia, but the primary (idiopathic) autoimmune haemolytic anaemia (AIHA) cannot be distinguished from secondary immune mediated anaemia (IMHA) using this test, which is caused by microbial agents or drugs.

A comparable direct test for the detection of antibodies against thrombocytes in patients with idiopathic thrombocytopenia has also been developed, however it is not commonly available for routine diagnosis.

Tests for the detection of other antibodies, such as antibodies against the acetylcholine receptor, for the diagnosis of myasthenia gravis, or antibodies against thyroglobulin and thyroid peroxidase for diagnosis of autoimmune hypothyroidism, are not commonly available.

Immunohistochemistry techniques are very useful methods for the detection of free or immune complex-bound autoantibodies in biopsy specimens. Those are usually used for the detection of skin autoantibodies, which can distinguish between various types of pemphigus complexes. The use of these methods for the detection of various types of glomerulonephritis, or inflammatory bowel disease, is less common but likewise significant.

A Non-specific Immunological Profile Testing:

Laboratory assessment of primary or secondary immunodeficiency is demanding for both methodical background and financial costs and especially tests of cell activities are offered only by specialised laboratories that are usually associated with universities or research institutions.

Immunological laboratories have different validated methods available (Table 1); nevertheless, it is always necessary to perform a set of immunological examinations. An isolated finding of a decrease in one of the immunological parameters determined, does not necessarily give evidence of immunodeficiency. Also, if the values of one or more parameters are changed, it is recommended to repeat the examination to confirm persistent immunodeficiency.

Table 5. Methods of immunological profile.

Parameter	Methods
Phagocytosis	Migration and chemotaxis under agarose, test of synthetic particles or microbes ingestion, chemiluminescence, detection of respiratory burst, microbicidity test
Lymphocyte subsets	Flow cytometry, immunohistochemistry
Lymphocyte activity	Lymphocyte transformation test, mixed lymphocyte reaction
Cytokines	Bioassay, ELISA, PCR
Immunoglobulin levels	Single radial immunodiffusion RID, ELISA
Complement	Haemolytic activity, ELISA
CRP, lysozyme and other humoural factors	ELISA, turbidimetry

Cell counts and activity detection follow up the haematological analysis of total and differential leukocyte counts. It occurs in the assessment of functional activity of phagocytic cells and lymphocytes, and sometimes also in lymphocyte subset identification (see below). Phagocytic activity may be studied by means of a number of tests (Table1); however, their diagnostic values vary. The lymphocyte transformation test (LTT) which monitors capability of lymphocyte stimulation, by non-specific mitogens for cell proliferation, is most valuable, however technically demanding. The most common findings in immunosuppression of animals are lymphopenia, together with dysbalance of lymphocyte subsets and a decreased activity of lymphocytes in LTT.

These changes were detected in primary immunodeficiencies (e.g., severe combine immunodeficiency disease) and also in German shepherd deep pyoderma, demodicosis, distemper, parvovirosis, in cats in FIV, FeLV and FIP infections, and also in chronic renal failure.

Among the tests of humoural factors, the **detection of total concentration of antibody isotypes is crucial**. The radial immunodiffusion test is simple and available as a commercial kit. It is mostly used for the assessment of isotypes present in serum in high concentrations (IgG, IgM), whilst the ELISA method is preferred for the detection of isotypes present in serum in low concentrations (IgA, IgE). Reduced concentrations of immunoglobulins indicate humoural immunodeficiency, which may be primary (selective IgA deficiency is most common) or secondary (occurs after some infectious diseases, commonly of viral origin). Decreased levels are sometimes found in newborns with non-sufficient colostrum supply. Elevated levels of immunoglobulins are found in chronic inflammatory processes and infections. Extremely high levels of serum immunoglobulins are detected in myeloma and occasionally in some infectious diseases.

Level or activity of complement proteins are a significant immunological parameter too. It is assessed by the test of haemolysis of sensitised erythrocytes, or immunochemical serological analysis of the respective components, particularly C3.

Advanced Methods in Immunology:

Similarly, as in related fields, knowledge in the field of immunology has been enormously extended by application of methods monitoring events in the cell at molecular levels, using the methods of genomics and proteomics. Some of these methods have already been applied to clinical immunology, usually as newly obtained knowledge from clinical and experimental studies. However, due to the fact that they are technically demanding and thus expensive, they are only rarely used for direct diagnosis.

Flow cytometry is the most commonly and recent method used, for the determination of lymphocyte subsets. Lymphocytes appear to be in a uniform population when viewed by microscopy. However, they are divided functionally into a series of subpopulations, which undertake various functions (Table 2). Monoclonal antibodies against phenotypic surface molecules are used for distinguishing between respective subtypes of lymphocytes. Changes in T and B lymphocyte ratios and changes in the ratios of helper (Th) and cytotoxic (Tc) T lymphocytes have been studied intensively. Changes in the ratios of these cells have been found to be indicative of SLE, GSP-associated immunodeficiency or leishmaniosis. More recently, other minority subsets (γδ T cells, NK cells), are studied in dogs in connection with immune diseases. Flow cytometry is also exploited, such as when detecting cytoplasm proteins including cytokines, distinguishing between apoptosis and necrosis and determining cell cycle stages, which is a test used in oncology diagnoses.

Table 6. Lymphocyte subsets in dogs.

Subtype	Phenotypic molecules	Range in blood
T helper cells (Th)	CD3, CD4, TCRαβ, (CD2, CD5)	30-48 %
T cytotoxic cells	CD3, CD8, TCRαβ, (CD2,	15-25 %

(Tc)	CD5)	
B lymphocytes	CD19, CD21, sIgM	12-25%
γδ T cells	CD3, CD8±, TCRγδ	1-2 %
double positive T cells	CD3, CD4, CD8	0-1 %

Methods of molecular biology and genomics are even more noteworthy. Detection of gene expressions based on polymerase chain reaction techniques (at present it is above all reverse transcription-PCR, real-time PCR) are used in clinical immunology, above all for the cytokines detection. The most commonly detected cytokines are inflammatory cytokines (IL-1, IL-6, TNFα), regulating cytokines (Th1 cytokines--IL-2, IFNγ, Th2 cytokines--IL-4, IL-5, IL-10 and others) and chemokines. So far, the detection of cytokine in mRNA levels has been used in cells or tissues in dogs with various diseases including immune-mediated.

References:

CASTELLI MS, MCGONIGLE P, HORNBY PJ. THE PHARMACOLOGY AND THERAPEUTIC APPLICATIONS OF MONOCLONAL ANTIBODIES. PHARMACOL RES PERSPECT (2019) 00:E00535. DOI: 10.1002/PRP2.535 [DOI] [PMC FREE ARTICLE] [PUBMED] [GOOGLE SCHOLAR]

CHAN EK, FRITZLER MJ, WIIK A, ANDRADE LE, REEVES WH, TINCANI A, ET AL. THE IUIS/WHO/AF/CDC COMMITTEE FOR THE STANDARDIZATION OF AUTOANTIBODIES IN RHEUMATIC AND RELATED DISEASES - AUTOABSC.ORG - AUTOANTIBODY STANDARDIZATION COMMITTEE IN 2006. AUTOIMMUN REV (2007) 6:577–80. DOI: 10.1016/J.AUTREV.2007.05.001 [DOI] [PUBMED] [GOOGLE SCHOLAR]

CHAN EKL, DAMOISEAUX J, CARBALLO OG, CONRAD K, CRUVINEL WM, FRANCESCANTONIO PLC, ET AL. REPORT OF THE FIRST INTERNATIONAL CONSENSUS ON STANDARDIZED NOMENCLATURE OF ANTINUCLEAR ANTIBODY HEP-2 CELL PATTERNS 2014-2015. FRONT IMMUNOL (2015) 6:412. DOI: 10.3389/FIMMU.2015.00412 [DOI] [PMC FREE ARTICLE] [PUBMED] [GOOGLE SCHOLAR]

CLARK G, STOCKINGER H, BALDERAS R, VAN ZELM MC, ZOLA H, HART D, ET AL. NOMENCLATURE OF CD MOLECULES FROM THE TENTH HUMAN LEUCOCYTE DIFFERENTIATION ANTIGEN WORKSHOP. CLIN TRANSL IMMUNOL (2016) 5:E57. DOI: 10.1038/CTI.2015.38 [DOI] [PMC FREE ARTICLE] [PUBMED] [GOOGLE SCHOLAR]

DAMOISEAUX J, ANDRADE LEC, CARBALLO OG, CONRAD K, FRANCESCANTONIO PLC, FRITZLER MJ, ET AL. CLINICAL RELEVANCE OF HEP-2 INDIRECT IMMUNOFLUORESCENT PATTERNS: THE INTERNATIONAL CONSENSUS ON ANA PATTERNS (ICAP) PERSPECTIVE. ANN RHEUM DIS (2019) 78:879–89. DOI: 10.1136/ANNRHEUMDIS-2018-214436 [DOI] [PMC FREE ARTICLE] [PUBMED] [GOOGLE SCHOLAR]

ENGEL P, BOUMSELL L, BALDERAS R, BENSUSSAN A, GATTEI V, HOREJSI V, ET AL. NOMENCLATURE 2015: HUMAN LEUKOCYTE DIFFERENTIATION ANTIGEN WORKSHOPS AS A DRIVING FORCE IN IMMUNOLOGY. J IMMUNOL (2015) 195:4555–63. DOI: 10.4049/JIMMUNOL.1502033 [DOI] [PUBMED] [GOOGLE SCHOLAR]

POMÉS A, DAVIES JM, GADERMAIER G, HILGER C, HOLZHAUSER T, LIDHOLM J, ET AL. WHO IUIS ALLERGEN NOMENCLATURE SUB-COMMITTEE. WHO/IUIS ALLERGEN NOMENCLATURE: PROVIDING A COMMON LANGUAGE. MOL IMMUNOL (2018) 100:3–13. DOI: 10.1016/J.MOLIMM.2018.03.003 [DOI] [PMC FREE ARTICLE] [PUBMED] [GOOGLE SCHOLAR]

PROHÁSZKA Z, NILSSON B, FRAZER-ABEL A, KIRSCHFINK M. COMPLEMENT ANALYSIS 2016. IMMUNOBIOLOGY (2016) 221:1247–58. DOI: 10.1016/J.IMBIO.2016.06.008 [DOI] [PUBMED] [GOOGLE SCHOLAR]

STADLER C, SKOGS M, BRISMAR H, UHLÉN M, LUNDBERG E. A SINGLE FIXATION PROTOCOL FOR PROTEOME-WIDE IMMUNOFLUORESCENCE LOCALIZATION STUDIES. J PROTEOMICS (2010) 73:1067–78. DOI: 10.1016/J.JPROT.2009.10.012 [DOI] [PUBMED] [GOOGLE SCHOLAR]