# Cold Agglutinin test

A cold agglutinin test may be used to help detect cold agglutinin disease and determine the cause of a person's [hemolytic anemia](https://labtestsonline.org/understanding/conditions/anemia/start/6). It may be used as a follow-up test after a [complete blood count (CBC)](https://labtestsonline.org/understanding/analytes/cbc/) shows a decrease in a person's [red blood cell (RBC) count](https://labtestsonline.org/understanding/analytes/rbc/) and [hemoglobin](https://labtestsonline.org/understanding/analytes/hemoglobin/), especially if these findings are linked to an exposure to cold temperatures.

Cold agglutinin disease is a rare [autoimmune disorder](https://labtestsonline.org/understanding/conditions/autoimmune/) in which [autoantibodies](https://labtestsonline.org/understanding/analytes/autoantibodies/) produced by a person's [immune system](https://labtestsonline.org/glossary/immune-system/) mistakenly target and destroy RBCs, causing hemolytic anemia. These autoantibodies are cold-reacting and can cause signs and symptoms related to [anemia](https://labtestsonline.org/understanding/conditions/anemia/start/1) after an affected person is exposed to cold temperatures. This disease may be classified as either primary or secondary, triggered by an infection or other condition.

This test is performed on a series of dilutions of a blood sample that are prepared in test tubes and then cooled to a low temperature, typically 4°C (39.2°F). Each dilution is then evaluated for clumped RBCs, with the results reported as a [titer](https://labtestsonline.org/glossary/titer/) – the highest dilution that shows detectable clumping. When cold agglutinins are present, RBC clumping is visible while the sample is cold and disappears when the sample is warmed. The exact temperature at which this will occur will vary as some cold agglutinins will react over a wider temperature range than others.

**When is it ordered?**

This test may be ordered when a person has reactions to cold temperature exposures and has signs of [hemolytic anemia](https://labtestsonline.org/understanding/conditions/anemia/start/6) that may be due to cold agglutinin disease. Symptoms may include:

* Fatigue, weakness, lack of energy, pale skin (pallor), dizziness and/or headaches from anemia
* In some cases, painful bluish fingers, toes, ears, and the tip of the nose that occur with exposure to cold temperatures

**What does the test result mean?**

The result of a cold agglutinin test is typically reported as a [titer](https://labtestsonline.org/glossary/titer/), such as 1:64 or 1:512. A higher number means that there are more autoantibodies present. Higher titers of autoantibodies and those that react at warmer temperatures are associated with [hemolytic anemia](https://labtestsonline.org/understanding/conditions/anemia/start/6) and worse symptoms.

The degree of red blood cell (RBC) [hemolysis](https://labtestsonline.org/glossary/hemolysis/) and hemolytic anemia will vary from person to person and with each episode of cold exposure. Some conditions, such as [infectious mononucleosis](https://labtestsonline.org/understanding/analytes/mono/tab/sample), are frequently associated with elevated cold agglutinins but rarely associated with anemia.

# Monospot test

Epstein-Barr virus (EBV) infection induces specific antibodies to EBV and various unrelated non-EBV heterophile antibodies. These heterophile antibodies react to antigens from animal RBCs.

* Sheep RBCs agglutinate in the presence of heterophile antibodies and are the basis for the Paul-Bunnell test.
* Agglutination of horse RBCs on exposure to heterophile antibodies is the basis of the Monospot test.

Heterophile test antibodies are sensitive and specific for EBV heterophile antibodies, they are present in peak levels 2-6 weeks after primary EBV infection, and they may remain positive in low levels for up to a year.

The latex agglutination assay, which is the basis of the Monospot test using horse RBCs, is highly specific. Sensitivity is 85%, and specificity is 100%.

The heterophile antibody test (eg, the Monospot test) results may be negative early in the course of EBV infectious mononucleosis. Positivity increases during the first 6 weeks of the illness. Patients who remain heterophile negative after 6 weeks with a mononucleosis illness should be considered as having heterophile-negative infectious mononucleosis.

* Patients with heterophile infectious mononucleosis should be tested for EBV-specific antibodies before definitively diagnosing heterophile-negative infectious mononucleosis.
* Patients with heterophile- or Monospot-negative infectious mononucleosis should be tested serologically as are patients who present with a mononucleosis like illness who are negative for heterophile antibodies. The heterophile test is less useful in children younger than 2 years, in whom the results are frequently negative.
* Although virtual 100% specificity exists with the Monospot test, rarely, other disorders have been reported that may produce a false-positive Monospot test result. These causes of false-positive Monospot test results include toxoplasmosis, rubella, lymphoma, and certain malignancies, particularly leukemias and/or lymphomas.

Testing for EBV-specific antibodies is as follows:

* EBV induces a serological response to the various parts of the Epstein-Barr viral particle. IgM and IgG antibodies directed against the VCA of EBV are useful in confirming the diagnosis of EBV and in differentiating acute and/or recent infection from previous infection. EBV IgM VCA titers decrease in most patients after 3-6 months but may persist in low titer for up to 1 year. EBV IgG VCA antibodies rise later than the IgM VCA antibodies but remain elevated with variable titers for life.
* False-positive VCA antibody titer results may occur on the basis of cross-reactivity with other herpes viruses, eg, CMV, or with unrelated organisms, eg,*Toxoplasma gondii.*

Other antigens indicating EBV infection are less useful diagnostically and include early antigen (EA), which is present early in EBV infectious mononucleosis. EBV nuclear antigen (EBNA) appears after 1-2 months and persists throughout life. The presence of elevated EBNA titers has the same significance as elevated IgG VCA titers. The presence of these antibodies suggests previous exposure to the antigen (past infection) and excludes EBV infection acquired in the previous year.

As with heterophile antibody responses, specific EBV antibodies may not be present in children younger than 2 years.

Heterophile antibody tests

* Patients with infectious mononucleosis should first be tested with a heterophile antibody test. The most commonly used is the latex agglutination assay using horse RBCs, and it is marketed as the Monospot test. Enzyme-linked immunosorbent assay (ELISA) rapid diagnostic tests are also available, which are based on the detection of heterophile antibodies. Physicians should remember that heterophile antibody responses require 1-2 weeks to become positive. In a group of patients with EBV mononucleosis, the number of patients becoming positive increases to a maximum 6 weeks after the onset of the illness.
* If results are initially negative, a Monospot test should be ordered weekly for 6 weeks in patients with suspected EBV infectious mononucleosis. If the Monospot test remains persistently negative after 6 weeks of weekly serial testing, then a specific EBV serological test should be ordered. Before patients with an infectious mononucleosis–like syndrome are labeled as having heterophile-negative infectious mononucleosis, specific EBV serological tests should be obtained, and the results should be negative

# Enzyme-Linked Immunosorbent Assay (ELISA)

**Enzyme-linked immunosorbent assay,** commonly known as **ELISA** (or EIA), is similar in principle to RIA but depends on an enzyme rather than a radioactive label. An enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. Such a substrate is called **a chromogenic substrate.** A number of enzymes have been employed for ELISA, including alkaline phosphatase, horseradish peroxidase, and β-galactosidase. These assays approach the sensitivity of RIAs and have the advantage of being safer and less costly.

There Are Numerous Variants of ELISA

## INDIRECT ELISA

Antibody can be detected or quantitatively determined with an indirect ELISA (Figure (26)). Serum or some other sample containing primary antibody (Ab1) is added to an antigen- coated microtiter well and allowed to react with the antigen attached to the well. After any free Ab1 is washed away, the presence of antibody bound to the antigen is detected by adding an enzyme-conjugated secondary anti-isotype antibody (Ab2), which binds to the primary antibody. Any free Ab2 then is washed away, and a substrate for the enzyme is added. The amount of colored reaction product that forms is measured by specialized spectrophotometric plate readers (ELISA reader)

Indirect ELISA is the method of choice to detect the presence of serum antibodies against human immunodeficiency virus (HIV), the causative agent of AIDS. In this assay:

* 1. The virus is grown in vitro in a human T-cell culture.
  2. Purified whole virus is disrupted and viral proteins are immobilized onto plastic beads or multiwell trays.
  3. Antibodies to any of these antigens will bind to them and be immobilized.
  4. Excess serum proteins are removed by washing the beads (or wells) and an enzyme-linked anti-human gamma globulin antibody is added. The presence of this second antibody can be detected calorimetrically by adding a substrate for the enzyme that will yield a colored end product.
  5. The rate of substrate degradation is determined by the amount of enzyme-labeled antibody that is bond, which is directly proportional to the amount of antigen in the solution being tested. A substrate that gives a color change when degraded is chosen. The color change can be measured quantitatively in a spectrophotometer.



Figure (26): Indirect ELISA

Sandwich ELISA.

This assay uses two antibodies and is performed as follows.

* 1. The first antibody (e.g., antibody specific for HBsAg is coated on a plastic surface (polystyrene plate). The solution being tested for HBsAg is then applied to the surface. Any unreacted material is removed by washing.
  2. The second antibody (i.e., enzyme-labeled anti-HBsAg-specific antibody) is then applied. Any excess conjugate is removed by washing. Finally, the enzyme substrate is added to detect the presence of the enzyme.



Figure (27): Sandwich ELISA.

## Competitive ELISA

1. In this technique, antibody is first incubated in solution with a sample containing antigen.
2. The antigen-antibody mixture is then added to an antigen-coated microtiter well. The more antigen present in the sample, the less free antibody will be available to bind to the antigen-coated well.
3. Addition of an enzyme-conjugated secondary antibody (Ab2) specific for the isotype of the primary antibody can be used to determine the amount of primary antibody bound to the well as in an indirect ELISA.
4. In the competitive assay, however, the higher the concentration of antigen in the original sample, the lower the absorbance.



Figure (28): Competitive ELISA.