

Microbiological Diagnostics: Serological Methods

Overview of serological diagnostic techniques used to detect infections. These methods rely on the specific interaction between antigens and antibodies and are widely used in microbiology and clinical diagnostics.

1.5 Serological Diagnostic Methods

Serological reactions are *in vitro* diagnostic methods based on the specific binding between antigens and antibodies. The term serology originates from the word serum, because antibodies involved in these reactions are present in serum.

In diagnostics we may detect either:

- the antigen of the pathogen
- or the specific antibody produced against it.

Direct antigen detection may be performed from patient samples or from cultured microbes. In cases where the pathogen is difficult to culture, detection of antibodies in blood or cerebrospinal fluid is used to confirm infection.

1.5.1 Serological Reactions Visible to the Naked Eye

In these reactions antigens and antibodies form lattice-like complexes that are visible without magnification. Antibodies are at least bivalent and antigens are typically multivalent.

Depending on the size of the antigen-bearing particles, three main reaction types exist:

- Agglutination
- Flocculation
- Precipitation

Agglutination

During agglutination the antigen and/or antibody is located on a cell surface or on particles roughly the size of cells.

Slide agglutination can demonstrate the presence of specific antigens. When binding occurs, visible clumps appear on the slide against a clear background. The reaction is qualitative.

Examples of Agglutination Tests

Examples include identification of bacterial antigens using specific antisera:

- Escherichia coli O, H and K antigen typing
- Lancefield grouping of Streptococcus
- Latex agglutination tests for Staphylococcus aureus

These reactions help distinguish bacterial strains and support diagnostic identification.

Latex Agglutination Rapid Tests

Latex particles coated with antibodies can bind specific bacterial antigens. This allows rapid detection of pathogens directly from clinical samples such as cerebrospinal fluid in meningitis diagnostics.

Tube Agglutination

Serial dilutions of patient serum are mixed with antigen. The test can determine whether antibodies are present and also measure antibody concentration.

Titer

The titer is the highest dilution of serum that still produces a visible antigen-antibody reaction. Determining antibody titer at different time points can help confirm infection by comparing paired serum samples.

Examples of Classical Agglutination Reactions

- Gruber–Widal reaction: used in typhoid fever diagnostics
- Weil–Felix reaction: historically used for rickettsial infections
- Wright reaction: used in brucellosis diagnosis

Agglutination in Microtiter Plates

Agglutination can also be performed in microtiter plates. Agglutinated particles form a diffuse network pattern in the well, while non-agglutinated cells settle as a compact dot.

TPHA and TPPA Tests

Treponema pallidum hemagglutination (TPHA) and particle agglutination (TPPA) tests detect antibodies against *T. pallidum*, the causative agent of syphilis. Both qualitative and quantitative results can be obtained.

Hemagglutination and Hemagglutination Inhibition

Certain viruses bind to red blood cells and cause agglutination. If the patient's serum contains antibodies against the virus, this agglutination is inhibited. These reactions can be used for both qualitative and quantitative virus detection.

1.5.1.2 Flocculation

Flocculation occurs when soluble antigens and antibodies form visible flakes or precipitates in solution. The strongest reaction occurs near the equivalence zone, where antigen and antibody concentrations are balanced.

Flocculation reactions are used in some syphilis screening tests (e.g., RPR and VDRL).

1.5.1.3 Precipitation

Precipitation reactions occur when antibodies bind soluble antigens and form insoluble complexes. A classic example is the Elek test for detecting toxin production by *Corynebacterium diphtheriae*.

1.5.2 Serological Reactions Not Visible to the Naked Eye

In these reactions labeled antibodies or antigens are used to detect antigen-antibody binding. Unbound components are washed away to preserve specificity.

Immunofluorescence

Fluorescently labeled antibodies allow visualization of antigen-antibody binding under a fluorescence microscope.

Direct immunofluorescence detects antigens in samples.

Indirect immunofluorescence detects antibodies in patient serum.

ELISA and CLIA

ELISA (Enzyme■Linked Immunosorbent Assay) and CLIA (Chemiluminescence Immunoassay) are automated serological methods used to detect both antigens and antibodies.

Principle of ELISA and CLIA

In ELISA an enzyme converts a substrate into a colored product measured as optical density. In CLIA the enzyme produces a light-emitting reaction that can be measured by detectors.

These methods are widely used in diagnostics, for example in hepatitis B antigen detection or hepatitis A antibody testing.