

## Atomic absorption spectrophotometry

A technique of element content determination by atomizing an element sample by putting it in a chemical flame or in a heated graphite tube. To the atoms evaporated in a flame, an element specific light is emitted, and the amount of light absorbed is measured to determine the element content.

## Chemiluminescent enzyme immunoassay (CLEIA)

Antibodies linked to the solid phase are reacted with antigens that are secondarily reacted with enzyme-labeled antibodies. After adding a chemiluminescent substrate, the emission intensity is measured.

## Chemiluminescent immunoassay (CLIA)

Antibodies linked to the solid phase are reacted with antigens that are secondarily reacted with chemiluminescent-labeled antibodies. The emission intensity of chemiluminescent substance is measured.

## Chromogenic substrate method

By adding heparin, an antithrombin III (AT-III)-heparin complex is generated. Then, the thrombin inactivation capacity is measured using a chromogenic substrate for thrombin.

## Clotting time

Clotting time is the time required for a plasma sample to coagulate, using plasma without a factor to be tested, thromboplastin, actin, and calcium chloride.

## Colorimetry

After converting an analyte to a coloring substance, a visible wavelength is irradiated. The absorbance is measured, and color of the analyte is compared with that of the standard solution.

## Complement fixation (CF)

A technique based on the principle that complements bind with antigen-antibody complexes and that they mediate hemolysis.

When red blood cells coated with hemolysin (sensitized red blood cells) bind to complements, hemolysis occurs; however, in the existence of the antigen-antibody complexes, complements are used, resulting in no hemolysis. Based on the hemolytic reaction, the presence of a specific antibody is determined.

## Direct sequencing method

Direct sequence analysis using PCR-amplified DNA as a sequencing template.

## Electro chemiluminescence immunoassay (ECLIA)

Antibody-bound beads are reacted with antigens that are secondarily reacted with antibodies labeled with ruthenium-pyridine complexes, which promotes the electrochemical reaction. The emission intensity of ruthenium-pyridine complexes is measured.

## Electrophoresis

A technique for analysis of macromolecules based on the following phenomena: When an electric current is applied to an electrolyte solution in which charged particles suspended, the particles migrate towards the electrode of opposite charge. Based on the length of migration, the target molecule is determined.

Cellulose acetate membrane, agarose gel, and polyacrylamide gel are used as support media.

## Enzymatic method

This technique is based on the same assay principle as the colorimetry. Analytes are specifically measured using enzymatic reactions.

## Enzyme immunoassay (EIA)

EIA is based on the same assay principle as RIA. Using either enzyme-labeled antigen or antibody as a labeled-substance, the antigen-antibody reaction is carried out. After adding a chromogenic substrate, the enzyme activity is measured.

## Enzyme-labeled antibody method

A technique to measure the enzyme activity of a specific antigen. Using enzyme-labeled antibodies to the target antigen, the antigen-antibody reaction is carried out. After adding a chromogenic substrate, the enzyme activity is measured.

The direct method uses enzyme-labeled antibodies that react directly with the antigen. The indirect method uses unlabeled antibodies that react with the antigen first and enzyme-labeled antibodies for the secondary reaction.

## Enzyme-linked immunospot (ELISPOT)

In a plate coated with a specific antibody, isolated cells and a specific antigen are added and cultured. Proteins secreted by the cells are reacted with a chromogenic substrate to generate spots.

## Enzyme-linked immunosorbent assay (ELISA)

Antibodies linked to the solid phase are reacted with antigens that are secondarily reacted with enzyme-labeled antibodies. After adding a chromogenic substrate, the enzyme activity is measured.

# overview of methodology

## Flow cytometry

A technique for analysis of individual cells. Cells stained with fluorochrome-labeled monoclonal antibodies are flown in a stream of solution at a high speed and are passed through the laser beam. The cells are individually analyzed by forward scatter (size of the cell), 90° side scatter (internal complexity of the cell), and fluorescence intensity (cell surface expression of the antigen).

Two-color flow cytometry uses a combination of fluorochromes for double staining.

## Fluorescence enzyme immunoassay (FEIA)

One of the techniques of EIA. Using either enzyme-labeled antigen or antibody as a labeled-substance, the antigen-antibody reaction is carried out. After adding a fluorescence substrate, the fluorescence intensity is measured.

## Fluorescence *in situ* hybridization (FISH)

The target DNA is hybridized with a fluorescent probe. Using a light with a specific wavelength, the fluorescent site in the chromosome is detected as a signal by fluorescence microscope.

The direct method uses a fluorescent probe to directly bind to the target DNA. The indirect method uses a labeled probe to bind to the target DNA which is subsequently bound to a fluorescence substance for color development.

## Fluorescent antibody method (FA)

Using fluorochrome-labeled antibodies to the target antigen, the antigen-antibody reaction is carried out, and the fluorescence intensity is measured using fluorescence microscope.

The direct method uses fluorochrome-labeled antibodies that react directly with the antigen. The indirect method uses antibodies that react with the antigen first and then fluorochrome-labeled antibodies for the secondary reaction.

## Gene analysis by fluorescence correlation spectroscopy (gFCS)

A genetic analysis technique. Following PCR using fluorescence-labeled primers, the difference in the primer's molecular size is determined as a time-course difference in the fluorescence intensity (fluorescence fluctuation) by the single-molecule fluorescence detection system. Then, the analysis is performed using fluorescence correlation spectroscopy (FCS).

## Gold colloid method

An optical measurement method using color change developed by aggregates of colloidal gold particles after antigen-antibody reaction with gold colloid-labeled antibodies.

## Hemagglutination (HA)

Red blood cell surface antigens are reacted with the antibodies.

After the antigen-antibody reaction is completed, the presence of the antibody is determined based on appearance or non-appearance of clots.

## Hemagglutination inhibition (HI)

A technique based on the phenomenon that hemagglutinating activities of viruses are inhibited by antibodies against the viruses. Antigen-antibody complexes are reacted with red blood cells, and the presence of the antibody against the virus is determined by whether or not hemagglutination is inhibited or not.

## High performance liquid chromatography (HPLC)

A chromatography technique using a liquid mobile phase. HPLC separates a mixture into components promptly and accurately, using a column filled with a high-density absorbent and a high-pressure pump.

## Immunoradiometric assay (IRMA)

IRMA is one of the techniques of RIA. Antibodies linked to the solid phase are reacted with antigens that are secondarily reacted with antibodies labeled with radioactive isotope (RI).

This is also called as the sandwich technique, because the solid-phase linked antibodies and the RI-labeled antibodies bind to the antigens, respectively, forming an antigen sandwich.

## Indirect fluorescent antibody method (IFA)

Using fluorochrome-labeled antibodies to the target antigen, the antigen-antibody reaction is carried out, and the fluorescence intensity is measured using fluorescence microscope.

The direct method uses fluorochrome-labeled antibodies that react directly with the antigen. The indirect method uses antibodies that react with the antigen first and then fluorochrome-labeled antibodies for the secondary reaction.

## Invader method

A technique consisting of two steps of homogeneous isothermal reaction to detect genetic polymorphisms by the use of cleavase (one of the endonucleases) that specifically recognizes and cleaves the triple-stranded DNA structure.

## Ion-selective electrode

The electrode method uses electric charge-transfer reaction at the liquid interface.

Ion-selective electrode is used to measure ionic concentrations based on a potential difference which occurs proportionally to the logarithm of ionic activity in response to a specific ion.

## Isothermal nucleic acid amplification test

Unlike PCR, this method amplifies nucleic acids at constant temperature, using strand displacement DNA synthesis enzymes, etc.

## Latex agglutination (LA)

Using antigen- or antibody-coated latex particles (sensitizing latex particles), the antigen-antibody reaction is carried out. The existence of the antibody or antigen is determined based on appearance or non-appearance of clots.

## Latex agglutination immunoassay (LA)

Using antigen- or antibody-coated latex particles, the antigen-antibody reaction is carried out. The turbidity of clots formed by the antigen-antibody reaction is measured by the light transmittancy or light scattering intensity.

## Latex photometric immunoassay (LPIA)

Using antigen- or antibody-bound latex particles, the antigen-antibody reaction is carried out. The turbidity of clots formed by the antigen-antibody reaction is measured by the light transmittancy by irradiating a near infrared light.

## Line immunoassay

When antigens are mechanically applied onto a membrane, specific antibodies are reacted to the antigens, and antibodies labeled with enzymes are subsequently reacted to detect the antibodies.

## Liquid chromatography/mass spectrometry (LC/MS)

HPLC is a technique to separate a target substance quickly and accurately, which is combined with a mass spectrometer as a detector, thereby realizing further improvement in the detection selectivity and the qualitative function.

## Liquid chromatography-tandem mass spectrometry (LC/MS/MS)

Liquid chromatography separates the target substance into compounds using difference in the affinity, which is combined with the mass spectrometry function that further divides the compounds by mass difference to isolate, fragment, and detect a specific mass ion.

## Liquid (nucleic acid) hybridization

After isolating rRNA in a liquid phase, hybridization is performed using a chemiluminescent-labeled DNA probe. After allowing the hybrid to be attached to the separating agent, chemiluminescence detection is performed.

## Loop-mediated isothermal amplification (LAMP)

LAMP employs 4 kinds of primers that recognize 6 distinct sequences of a target gene. By using the chain substitution reaction, the reaction process proceeds at a constant temperature.

## MALDI-TOF-MS

An ionization technique that uses laser energy absorbing matrix to create ions by irradiating a laser to an analyte mixture without decomposing proteins is known as matrix-assisted laser desorption/ionization (MALDI). MALDI is combined with a technique of time-of-flight mass spectrometry (TOF-MS) which is based on the principle that the ion's time of flight changes depending on the formed ion's mass-to-charge ratio.

MALDI: Matrix-assisted laser desorption/ionization  
TOF-MS: Time-of-flight mass spectrometry

## Multiplex ligation-dependent probe amplification (MLPA)

A specific probe coupled with a common PCR primer sequence is hybridized to the target sequence. After PCR reaction, the amplified products are examined for their quantitative changes, especially for comparatively major genomic deletions and duplications.

## Multiplex PCR Fragment Analysis

After amplifying multiple target genes, capillary electrophoresis is performed on PCR amplicons by sequencer. Separated according to DNA size, the amplified peaks are analyzed and identified by the dedicated software with the predetermined QMVR width as a reference.

## Nephelometry

A technique to determine the light-scattering intensity by irradiating light to a turbid substance formed by an antigen-antibody reaction.

## Neutralization test (NT)

A technique based on neutralization: Through reaction of a virus with specific antibody, loss of infectivity occurs. The virus and the antibody against the virus are reacted and inoculated into cell culture that is sensitive to the virus. The presence of neutralizing antibody is determined based on the cytopathogenic effect (CPE).

## Next generation sequence (NGS) method

A method for simultaneous determination of nucleotide sequences of a large amount of DNA fragments using a next generation sequencer.

## Ouchterlony method

### Ouchterlony double immunodiffusion

One of the gel-diffusion techniques, also called as ouchterlony double immunodiffusion, to identify antigen and antibody reactions. After antibodies and antigens are diffused in the gel plate, the presence or absence and the number of precipitin lines formed by the antigen-antibody reaction are confirmed.

# overview of methodology

## Particle agglutination (PA)

Using antigen- or antibody-coated gelatin particles (sensitizing particles), etc., the antigen-antibody reaction is carried out. The existence of the antibody or antigen is determined based on appearance or non-appearance of clots.

## Passive hemagglutination (PHA)

The surface of red blood cells is coated with antigen to form sensitized red blood cells. Using the sensitized red blood cells, the antigen-antibody reaction is carried out. The presence of the antibody is determined based on appearance or non-appearance of clots.

## Polymerase chain reaction (PCR)

A technique to amplify a specific target region of DNA in an exponential manner. By heating DNA molecules, the DNA denatures and becomes single stranded, and then by cooling, the DNA anneals to the double stranded again. By using this phenomenon, a single-stranded DNA is used as a template to bind the target primer. The DNA synthesis occurs by repeated cycles of DNA polymerase activity.

## Radioimmunoassay (RIA)

A target antigen is labeled with radioactive isotope (RI) and bound to its specific antibodies which is then competitively reacted with an antigen in a specimen. After the antigen-antibody reaction, the bound labeled antigens (bound) are separated from the unbound ones (free), and the antigen concentration is determined based on the proportion of radioactivity.

To separate the bound and free antigens (B/F separation), the following methods are used. Solid-phase method: Antibodies are linked to the solid phase. Double antibody method: Antigen-antibody complexes are bound to the second antibody and precipitated. Ammonium sulfate precipitation method: Antigen-antibody complexes are precipitated by ammonium sulfate. PEG method: Antigen-antibody complexes are precipitated by a precipitation reagent.

## Radioreceptor assay (RRA)

RRA is based on the same assay principle as RIA. Instead of an antibody, a receptor is used, and the biological activity is determined by the reactivity.

## Real-time PCR

Real-time PCR is the one of the nucleic acid amplification tests using polymerase chain reaction (PCR) as the basic principle. By the use of oligonucleotide that yields fluorescence after degradation, the fluorescent signal is identified for each PCR cycle, which enables the real-time quantification of the target nucleic acid.

## Reverse transcriptase-polymerase chain reaction (RT-PCR)

When RNA to be amplified by PCR, complementary DNA (cDNA) is synthesized using reverse transcriptase (RT) using RNA as a template.

## Southern blot hybridization

A technique to detect a specific gene by the following procedures: After restriction enzyme digestion of DNA, DNA is isolated and denatured to a single-stranded form with electrophoresis. Using capillary action, the single-stranded DNA is transferred to a nylon membrane and hybridized with a target probe to detect the target gene. This technique is used for analysis of abnormal changes in DNA in a quantitative and qualitative manner.

## Transcription-mediated amplification (TMA)

A technique of RNA amplification using two kinds of enzymes, two kinds of primers, and substrates. Extracted RNA allows reverse transcriptase to create double-stranded DNA. Using the double-stranded DNA as a template, RNA synthesis is repeated by RNA polymerase transcription to amplify the target region of RNA.

## Transcription Reverse Transcription Concerted reaction (TRC)

A method for real-time detection of RNA amplified in one step by combining a DNA probe labeled with intercalating fluorescent dye and constant temperature RNA amplification.

## Turbidimetric immunoassay (TIA)

A technique to determine the light transmittancy by irradiating light to a turbid substance formed by an antigen-antibody reaction.

## Ultracentrifugation

A technique to separate one type of material from others and to measure physical properties based on difference in the specific gravity of proteins using an ultracentrifuge.

## Ultraviolet absorption spectrophotometry (UV)

This technique is based on the same assay principle as the colorimetry. Analytes are measured using ultraviolet wavelengths. Normally, near-ultraviolet wavelengths between 200 and 400 nm are used.

## Various types of bandings

Using cultured lymphocytes or bone marrow cells, cells in the metaphase are fixed. Then, a stain gives a series of light and dark stripes (bands) along the chromosome, and analysis is performed based on the distribution and light-and-dark differences of the stripes.

G-banding uses treatment with trypsin solution and Giemsa staining. C-banding consists of steps including HCl treatment, exposure to  $\text{Ba}(\text{OH})_2$ ,  $2 \times \text{SCC}$ , and Giemsa staining. Q-banding uses a stain called quinacrine mustard and the fluorescence pattern is observed using fluorescence microscopy. In high-resolution banding, visualized images of cell division between the end of the prophase and the beginning of the metaphase are used so that an increased number of bands are observed than in normal conditions.

## Western blot

A technique to detect a specific protein by separating proteins using electrophoresis, electrical transfer of proteins to a nitrocellulose membrane, and marking the target protein using a proper primary antibody and a proper enzyme-labeled secondary antibody. This technique is also called as immunoblot.

## $^3\text{H}$ -thymidine (TdR) uptake assay

A technique based on the phenomenon of lymphocyte blastogenesis after stimulation by nonself antigens.

Lymphocytes are cultured with a stimulant and  $^3\text{H}$ -thymidine. After the DNA synthesis, the amount of  $^3\text{H}$ -thymidine uptake by the cells is determined as the radioactivity. The stimulants include PHA, Con-A, and drugs.