19.2.3 Immunology

Adjuvant

A material introduced with an antigen to augment its immunogenicity.

Adsorb

To collect on a surface usually by a monomolecular layer of reactant. Materials such as plastic, glass, or particles (latex, bentonite, cellulose, etc.) that are used for the removal of antibodies or antigens by immobilizing the appropriate reactant to their surface.

Affinity

Defines and quantitates the force of association between a single antibody and a single epitope of antigen; the association constant, *K*, for the immunochemical reaction:

$$\text{Antibody} + \text{Antigen} \_ \text{Antibody-Antigen}$$

which can be expressed mathematically as:

$$K = \frac{[Antibody\text{-}Antigen]}{[Antibody]\,[Antigen]}$$

Average Affinity Constant (*K*)

The average binding constant (*K*) of a population of antibody molecules.

Agglutination

The immunochemical-specific aggregation of particulate matter such as bacteria, erythrocytes, or other cells, or synthetic particles such as plastic beads coated with antigens or antibodies. Such aggregation usually is dependent primarily on surface reactions mediated either by antigens or by antibodies physically or chemically attached to the particulate surfaces; agglutination of clumping of the particles follows as a secondary immune reaction.

> Agglutination inhibition - A type of agglutination in which particulate and soluble antigen compete for soluble antibody. Soluble antigen in the test medium reacts first with the soluble antibody, inhibiting agglutination of

indicator particles. With viral hemagglutination inhibition assays, host antibodies resulting from a specific infection are the most common forms of agglutination inhibition assays. In this case, viral-specific antibodies block the sites on the virus that agglutinate erythrocytes.

Hemagglutination - Agglutination reactions in which the particles used are erythrocytes. Hemagglutination may be either direct, in which erythrocyte antigens are reactants, or indirect (passive) for coated antigen or, in the case of reverse (passive) assays, coated antibody. One of the most common uses of hemagglutination is to quantitate the number of hemagglutinating viruses (cf. influenza) or their soluble hemagglutinating surface subunits.

Indirect (passive) agglutination - The agglutination technique in which antigen first is coated artificially onto the particulate surfaces, either by physical absorption or chemical or immunochemical linkage. These antigen-laden particles then can be used to detect the presence of the corresponding specific agglutinins in test material. Agglutination results by cross-linking of the antigen-bearing particles onto an extensive antigen-antibody lattice (i.e., in detectable agglutination of the particles).

Mixed Agglutination Tests - The mixed agglutination phenomenon results from the reaction of antibody with similar antigenic determinants on two different types of particles (or cell types), with aggregation of these disparate particles. One particle is the indicator system for antibodies directed against and bound to the other particle or its surface antigen.

Reverse passive agglutination - In this modification of the indirect agglutination assay, particles are coated with antibody. These antibody-laden particles then become probes for detecting specific antigens in test material. Presence of the relevant antigen will result in agglutination of particles.

Antibody

The functional component of antiserum, often referred to collectively as a population of molecules, each member of which is capable of reacting with a specific antigenic determinant. An antibody molecule is, by definition, monospecific but might also be "idiospecific," "heterospecific," "polyspecific," or of "unwanted specificity." It cannot be "nonspecific" except in the sense of nonimmunochemical binding. These proteins are immunoglobulins and bind by means of specific binding sites to a specific antigenic determinant.

Antigen

Classically, a substance that will elicit the formation of antibodies in a suitable host. A more recent connotation defines an antigen as a substance that will combine with antibody through its antibody-binding sites.

Antigenic determinant

That part of the structure of an antigen molecule that is responsible for specific interaction with antibody molecules evoked by the same or a similar antigen.

Antiserum

A serum containing antibodies.

Avidity

Operationally defines the combined intensity of reactivities of an antiserum or antibody population. In effect, it represents the net affinity of all binding sites of all antibodies in the antiserum, under specified physicochemical reaction conditions. The avidity is a function of the affinities of the antibody-combining sites on all antibodies present in an antiserum and all the antigenic determinants of available macromolecules. Sometimes avidity can be expressed as an effective affinity constant.

Binding capacity

The capacity of a receptor to bind a ligand, expressed in operational units, unlike the quantitative mass units of the affinity constant.

Bound/free (B/F) ratio

The ratio of bound to free-labelled analyte in an immunoassay.

Carrier protein

A protein to which a specific ligand or hapten (see later) is conjugated. Also refers to unlabelled protein introduced into an assay at relatively high concentrations which distributes in a fractionation process in the same manner as a labelled protein analyte, present in very low concentrations. Also, a protein added to prevent nonspecific interaction of reagents with surfaces, sample components, and each other.

Complement

An array of serum proteins (some of which are enzymes) that become sequentially activated after the first member of the series is activated by either antigen-antibody complexes or microbial products.

Conjugate

A material produced by attaching two or more substances together. Conjugates of antibody with fluorochromes, radioactive isotopes, or enzymes are often used in immunoassays.

Cross Reactivity

The reaction of an antibody with an antigen other than that which elicited the formation due to the presence of related determinants.

Enzyme Conjugate

Designates a material that has an enzyme bound covalently.

Epitope

The minimum molecular structure ("antigenic determinant") that will react with an antibody and may be only a portion of antigen.

Hapten

A specific substance that interacts with specific antibody-combining sites of an antibody molecule, but is not immunogenic by itself.

Heterogeneous immunoassay

An immunoassay that requires the physical separation of free-labelled antigen (or antibody) from labelled antigen (or antibody) bound in an immune complex, prior to measurement of the quantity of label.

Homogeneous immunoassay

An immunoassay in which no physical separation is performed. The specific activity of the label or the signal is modulated according to the analyte content of the sample.

Hybridoma

A cell derived from the fusion between a *B* cell and a plasmacytoma.

Immunoassay

A ligand-binding assay that uses a specific antigen or antibody capable of binding to the analyte.

Immunogen

A substance that elicits a cellular immune response and/or antibody production.

Immunoglobulin

A glycoprotein found in serum or other body fluids possessing antibody activity.

> Immunoglobulin class - A classification of immunoglobulin based on antigenic and structural differences of the Heavy Peptide Chain. There are five classes: IgG, IgA, IgM, IgD, and IgE.
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> Immunoglobulin Subclass - A subdivision of the classes based on structural and antigenic differences in the Heavy Peptide Chain; Isotype. Four human IgG subclasses and two IgA subclasses have currently been recognized; IgM subclasses have been postulated; IgD and IgE subclasses are unknown.

Immunoprecipitation Analysis

Any analysis that relies on a system that analyzes a precipitate formed between antibody and antigen.

Label

An easily detected substance that is attached to a reagent in an immunoassay. The assay signal is either a measurable property of the label or is produced by the label. In enzyme immunoassay (EIA) the label is the enzyme; in fluorescence immunoassay (FIA) the label is a fluorescent material; in radioimmunoassay (RIA) the label is the radionuclide.

Ligand

A substance or part of a substance that binds to a specific receptor.

Matrix

The milieu of the sample (e.g., serum) containing the analyte. The matrix can influence the behaviour of an immunoassay due to specific (direct) and nonspecific (indirect) interferences.

Monoclonal

Arising from a single clone of cells, in the case of immunoglobulin, refers to its origin; usually the monoclonal antibody is of a single immunoglobulin class containing only one light chain type of either the K or L variety. Also refers to all antibody molecules having identical physical-chemical characteristics and antibody specificity. Monoclonal antibodies have very restricted structural diversity and are homogeneous compared with polyclonal antibodies.

Monospecificity

Monospecificity is functionally defined as the immunoreactivity of an antiserum with its designated antigen (e.g., antihuman IgG, antihuman IgG Fe piece, human IgG3 Fe piece, etc.). In practice, true monospecificity to naturally occurring antigens does not occur in antisera produced by the immunization of the intact animal. An attempt is made to reduce the level of unwanted specificities below that which will interfere with the intended use of a particular immunochemical test.

Nonradioisotopic Immunoassay

A type of immunoassay in which the antigen-antibody reaction is measured through the light-scattering properties of immune complexes or through the use of marker molecules attached to constituents of the immune reaction.

> Enzyme immunoassay (EIA) - A generic term for an immunoassay in which the analyte content of the sample is estimated by measuring the catalytic activity of a specific enzyme conjugate on a substrate.
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> Enzyme-linked immunosorbent assay (ELISA) - A heterogeneous enzyme immunoassay method where an antigen or antibody is firmly attached to a solid support.

Fluorescence immunoassay (FIA) - A generic term for an immunoassay in which the analyte is measured by fluorescence. This type of assay is carried out by conjugating fluorescent compounds to the antigen or antibody and then measuring the fluorescence in the antigen-antibody reaction.

Light-scattering immunoassay - A type of immunoassay which involves the detection of the antigen-antibody complex formation in an immune reaction by changes in turbidity (turbidimetry) or light scattering (nephelometry) in a fluid medium.

Polyclonal

Arising from different clones. A typical antiserum obtained from a conventional immunization is polyclonal.

Potency

The characteristic of an antibody representing the concentration (titre) of antibody and the avidity for a given substrate (antigen) in the defined method.

Radioligand assay

A technique in which unlabelled and radioactive labelled molecules of the same species compete for a limited number of binding sites on a specific binding protein. The binding protein may be an antibody, transport protein, hormone receptor, or any other cell-associated receptor or tissue component. The unlabelled ligand is the analyte. In the procedure, after a suitable reaction period, the bound ligand (both labelled and unlabelled) is separated from the free ligand, and the radioactivity of either fraction is measured. Calibration reference materials are included in the assay, and the concentration of unlabelled ligand can be estimated from the calibration curve or computed after application of a suitable curve-fitting routine.

Competitive protein binding assay - A type of radio-ligand assay in which the binding protein is a transport protein or enzyme.

Radioimmunoassay (RIA) - A type of radioligand assay in which the binding protein is an antibody.

Radioreceptor assay - A type of radioligand assay in which the binding protein is a hormone receptor.

Receptor

A specific molecule on the surface of a neutron or target cell that specifically binds to a specific molecule such as a neurotransmitter, hormone, antigen complement component, lymphokine, etc.

Sandwich immunoassay

An immunoassay using the chemical or immunochemical binding of the analyte to a solid phase and the immunochemical binding of a second (labelled) reagent to the analyte.

Specificity

An antiserum quality defining its reactivity with defined antigens. In a chemical context, the extent to which the assay responds only to (all subsets of) a specified analyte and not to other substances present in the sample.

Titre

The reciprocal of the dilution factor required to produce a defined outcome to a defined system. In a defined system it is usually proportional to the analyte concentration.