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PATENTS AND LITERATURE

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The objective of this section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. This issue on bioassays completes the five subject areas covered in 1985: immobilized biocatalysts; applied immunology; nucleic acid technology; affinity separations; and bioassays. Several new subject areas will be introduced in 1986: Enzymes in organic solvents and supercritical fluids; applications of polysaccharides; protein engineering; DNA probes for clinical applications; mammalian cell culture; and microbial transformations. In each issue, a new subject area will be introduced, with a review of recent patents and literature. The subject of the final Patents and Literature Section of 1985 is Bioassays.

BIOASSAYS

Patents

This section identifies and gives a brief description of patents from the US patent literature from January 1984 to September 1985. The major search heading was assay, with the cross-terms: immuno, enzym, and bio. The term enzyme electrode(s) was also searched. Both patent abstracts and titles were searched. Copies of US Patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

IMMUNOASSAYS

Abbott, S. D., and Luddy, M. A. G.
PARTICLE REAGENT SIZE DISTRIBUTION MEASUREMENTS FOR IMMUNOASSAY
US 4,521,521, Jun. 04, 1985
Assignee: E. I. Du Pont de Nemours and Company

A sensitive and rapid method is described for quantitatively assaying analytes in liquid media. Changes in particle size distribution of reagent particles containing insolubilized analyte caused by antibody-induced aggregation are directly measured. The amount of analyte initially present can be determined by measuring the change in particle size distribution with time.

Ali, M., Nalebuff, D., Fayemi, A., Ramanarayanan, M. P., and Mesa, T. R.
ENZYMATIC IMMUNOASSAY
US 4,454,226, Jun. 12, 1984

An enzyme immunoassay is described for detecting an antigen in a biologic fluid (or tissue) by contacting it with a specific antibody with either the fluid (or tissue) or the antibody having a solid component, contacting the resulting solid with a conjugate bindable with the antibody, and determining the enzyme activity of the resulting solid phase. The conjugate is peroxidase and an allergen (nonimmunoglobulin protein or primary amino group containing drug) having an average of 2–3 molecules of peroxidase per molecule of substance, with an average molecular weight of about 30,000. It is prepared by reacting peroxidase with phenyl isothiocyanate, oxidizing it to form aldehyde groups, and reacting it with the substance to form a Schiff's base, which is reduced to form a stable conjugate.

Auditore, H. K., and Miesowicz, F. M.
HOMOGENEOUS IMMUNOASSAY USING COVALENT HYBRID ANTIBODIES
US 4,446,233, May 01, 1984
Assignee: E. I. Du Pont de Nemours and Company

An immunoassay for antigens or haptens that utilizes covalent hybrid antibodies to modulate the activity of indicators, is described. The hybrid antibody has binding sites for the analyte and the indicator.

Berthold, F., and Kubisiak, H.
ASSAYS, INCLUDING IMMUNOASSAYS WITH FITC LABEL ACTIVATED BY SODIUM HYPOCHLORITE
US 4,435,509, Mar. 06, 1984
Assignee: Laboratorium Prof. Dr. Rudolf Berthold

A process is described for carrying out an analytical determination of the presence of a substance by means of chemiluminescence, employing fluorescein isothiocyanate as a labeling agent, triggering a chemiluminescence reaction by adding an aqueous solution of sodium hypochlorite and measuring the emission of light.

Brunhouse, R. F.
LABELED ANTIBODIES AND METHODS
US 4,490,473, Dec. 25, 1984
Assignee: PanAb

Sandwich immunoassays are described in which an anti-analyte antibody substituted with an *N,N,N*-trimethylammoniumphenyl group is coupled with a labeled antibody specific to *N,N,N*-trimethylammoniumphenyl.

Calenoff, E., Tsay, Y. G., Jones, R. M., and Scott, J. R.
FLUOROMETRIC ENZYME INHIBITION IMMUNOASSAY FOR MEASURING POTENCY OF ALLERGEN EXTRACTS
US 4,528,267, Jul. 09, 1985
Assignee: Axionics, Inc.

An inhibition assay is described for measuring the potency of allergen extracts in which a mixture of allergen extract and reference allergen specific IgE is incubated in a buffered solution with an insoluble support to which reference allergen is adhered. The conjugated IgE adhering to the insoluble support is reacted with an enzyme labeled anti-IgE antibody. The enzyme label is mixed with a substrate solution that yields a fluorescent product in the presence of the enzyme. The percentage of inhibition of the allergen specific IgE is determined from fluorescence levels measured for various extract concentrations.

Campbell, A. K., Simpson, J. S. A., and Woodhead, J. S.
DETECTING OR QUANTIFYING SUBSTANCES USING LABELING TECHNIQUES
US 4,478,817, Oct. 23, 1984
Assignee: The Welsh National School of Medicine

A method is described for detecting or quantifying a protein, antibody, antigen, hapten, hormone, metabolite, nucleic acid, or steroid in which the substance of interest is linked to a chemi- or bioluminescent label. A luminescent reaction is then triggered by the addition of an oxidizing agent or a catalyst, and the emitted light is observed. The luminescent reagent consists of antibodies labeled with a luminescent material, such as luminol. This reagent can be used to quantify antigens in an immunological assay.

Canfield, R. E., Ehrlich, P. H., and Moyle, W. R.
MONOCLONAL ANTIBODY MIXTURES AND USE THEREOF FOR ENHANCED SENSITIVITY IMMUNOASSAYS
US 4,514,505, Apr. 30, 1985
Assignee: The Trustees of Columbia University in the City of New York

Mixtures of at least two monoclonal antibodies that bind to different antigenic sites on the antigen and are capable of binding simultaneously to an antigen are useful in enhanced sensitivity assays for the antigen. By utilizing such mixtures in diagnostic assays for important antigens, enhanced sensitivity can be achieved, compared with assays employing individual monoclonal antibodies.

Carter, T. J. N., and Dahne, C.
ELLIPSOMETRICALLY MEASURING RATE OF OPTICAL CHANGE IN IMMUNOASSAY
US 4,508,832, Apr. 02, 1985
Assignee: Battelle Memorial Institute

A layer of bioactive molecules is coated on a dielectric substrate and is contacted with a solution to be analyzed containing a complex conjugate of this molecule. The rate of complexion of the conjugate with the layer, which is a function of its concentration in the analyte, is measured by optical means against a standard reference.

Chang, C. D., and Graham, H. A., Jr.
DOUBLE ANTIBODY CONJUGATE
US 4,433,059, Feb. 21, 1984
Assignee: Ortho Diagnostic Systems Inc.

An immunoassay is described with increased sensitivity and decreased complexity. A heterobifunctional coupling agent couples a univalent immunoglobulin specific for the surface antigens on erythrocytes to a second multivalent immunoglobulin that is specific for the antigen.

Chu, A. E.
SOLUBLE IMMUNOASSAY REAGENT COMPRISING LECTIN COVALENTLY BONDED TO REACTIVE COMPONENT
US 4,493,793, Jan. 15, 1985
Assignee: E–Y Laboratories

A lectin is covalently bound to an immunological conjugate, such as an antibody–antigen. Then, the lectin-conjugate is isolated from the reaction product mixture by one or more of the following: (1) reversible reaction of the lectin with an insolubilized sugar to isolate it from the remainder of the mixture; (2) reaction of one immunological component (e.g., antibody) bonded to the lectin with an insolubilized corresponding component (e.g., antigen) to separate it from the remainder of the mixture; and (3) filtration of the reaction components to separate them on the basis of product molecular weight.

Coate, S. R., and Binder, W. L.
ENZYME IMMUNOFLUORESCENT ASSAY FOR AUTOANTIBODIES
US 4,487,830, Dec. 11, 1984
Assignee: American Hoechst Corporation

A method is described for the determination of autoantibody in a test sample by contacting a substrate with sample; treating with labeled antihuman antibody selected from (1) a mixture of enzyme labeled antihuman antibody and fluorescent labeled antihuman antibody, and (2) antihuman antibody labeled with an enzyme and a fluorescent label; determining the enzyme activity of the treated substrate; and determining the immunofluorescent patterns in substrates exhibiting enzyme activity.

Cole, F. X.
IMMUNOASSAY WITH ANTIGEN OR ANTIBODY LABELED LIPOSOMES SEQUESTERING ENZYME
US 4,483,921, Nov. 20, 1984
Assignee: Collaborative Research, Inc.

An immunoassay method is described utilizing antigen tagged, enzyme encapsulating liposomes that are immunospecifically ruptured. A homogeneous phase reaction occurs with the antibody and the complement, acting to release the enzyme if an immunospecific antigen–antibody complex is formed at the surface of the liposome. The positions of the antigen and antibody can be reversed.

Cone, R. O., Jr., and Carpenter, C. R.
PREPRECIPITATED DOUBLE ANTIBODY IMMUNOASSAY METHOD
US 4,481,298, Nov. 06, 1984
Assignee: AMF Inc.

An immunoassay process is described for the detection of an antigen in a sample by: (a) forming a mixture of the sample with (1) a preformed complex of a high specificity primary antibody (present at low concentrations) and a secondary binding macromolecule (affinity purified and reacting with the Fc portion of the primary antibody) and with (2) a detectably labeled form of the antigen; (b) incubating the mixture (a) to allow competitive binding; and (c) detecting the separated complex or the separated suspension medium.

Craig, A. R., Frey, W. A., Leflar, C. C., Looney, C. E., and Luddy, M. A. G.
COVALENTLY BONDED HIGH-REFRACTIVE INDEX PARTICLE REAGENTS AND THEIR USE IN LIGHT SCATTERING IMMUNOASSAYS
US 4,480,042, Oct. 30, 1984
Assignee: E. I. Du Pont de Nemours and Company

Particle reagents for light scattering immunoassays are described, having high-refractive index shell-core polymers covalently bound to compounds of biological interest. The compounds of biological interest are determined measuring changes in turbidity caused by particle agglutination or its inhibition.

David, G. S., and Greene, H. E.
IMMUNOMETRIC ASSAYS USING MONOCLONAL ANTIBODIES
US 4,376,110, Mar. 08, 1983
Assignee: Hybritech, Inc.

A "two-site" or "sandwich" immunometric assay technique is described for determination of the presence and/or concentration of antigenic substances in fluids, using monoclonal antibodies. One monoclonal antibody is presented in a soluble labeled form and a second is presented bound to a solid carrier; the soluble and bound monoclonal antibodies may be the products of either the same or different cell lines.

Editorial Note: This early patent (above) has recently been ruled invalid in the US District Court, freeing others to pursue monoclonal-based diagnostics.

Deindoerfer, F. H., and Gangwer, J. R.
METHOD OF ANALYZING THE DISTRIBUTION OF A REAGENT BETWEEN PARTICLES AND LIQUID IN A SUSPENSION.
US 4,476,231, Oct. 09, 1984
Assignee: International Remote Imaging Systems, Inc.

An immunoassay procedure is described in which an analyte in a solution is reacted with a reagent with a label. The reagent is segregated into solid (particles) and a liquid phase. The unreacted reagent ion occupies one phase and the reacted reagent the other. An image of the suspension is then taken, converted into an electrical signal representation, stored in digital form, and processed by locating and quantifying the distribution of the labels.

Deutsch, A., Brandwein, H., Platt, H., Hunter, D. M., Dubitsky, A., and Durham, S. M.
ANTIGEN ASSAY METHOD AND KIT
US 4,477,576, Oct. 16, 1984
Assignee: Mex Research Associates

A method is described determining the concentration of an antigen in a sample by: (a) coating an antigen–protein conjugate onto a solid matrix; (b) conjugating an enzyme to an antibody specific for that antigen; (c) to a known quantity of solution (b), adding a specified quantity of a sample; (d) contacting the coated solid matrix (a) with solution (c) and incubating;

(e) removing the solid matrix from the solution and washing; (f) immersing the solid matrix in a solution containing a known amount of an enzyme-substrate to produce a product and then separating the solid matrix from the solution of enzyme-substrate; and (g) then measuring the amount of antigen in the sample against a standard.

Dreesman, G. R., and Kendall, C. E.
IMMUNOASSAY EMPLOYING MONOCLONAL HERPES SIMPLEX ANTIBODY AND BIOTIN–AVIDIN DETECTION SYSTEM
US 4,535,057, Aug. 13, 1985
Assignee: AMF Inc.

An immunoassay and kit for determining a viral antigen. The antigen is immunocaptured by an insoluble matrix containing IgG antibody, and the matrix is subsequently contacted by biotin labeled monoclonal IgM antibody and labeled avidin.

Fino, J. R., and Kirkemo, C. L.
SUBSTITUTED CARBOXYFLUORESCEINS
US 4,476,229, Oct. 09, 1984
Assignee: Abbott Laboratories

A method for determining ligands in biological fluids, using a novel class of tracer compounds employed as reagents in fluorescence polarization immunoassays, is described.

Foster, T. L., and Casey, R. C.
IMMUNOASSAY WITH ARTICLE HAVING SUPPORT FILM AND IMMUNOLOGICAL COUNTERPART OF ANALYTE
US 4,444,879, Apr. 24, 1984
Assignee: Science Research Center, Inc.

The apparatus is a water-insoluble article bearing a dried film of a synthetic, polymeric resin, having attached chemical groups capable of forming covalent bonds with immunoreactants, which can be applied to determine total immunoglobulin and IgE.

Frenzel, B.
CHEMILUMINESCENT IMMUNOASSAY WITH ACTIVATOR OF HYDROGEN PEROXIDE AND A CHLORAMINE
US 4,491,634 Jan. 01, 1985

A process is described for qualitative and quantitative determination of antigens, antibodies, and antigen–antibody complexes, using a chemiluminescent marker in a liquid- or solid-phase assay. The chemiluminescent marker and activator is selected from the combinations (a) hydrogen peroxide/chloramine–fluorescein, methylene blue, thionine (or equivalent chemiluminescent marker); and (b) calcium

hypochlorite–fluorescein (or equivalent derivative). The method is simpler, safer, and more sensitive than known methods.

Freytag, J. W.
IMMUNOASSAY WHEREIN LABELED ANTIBODY IS DISPLACED FROM IMMOBILIZED ANALYTE–ANALOG
US 4,434,236, Feb. 28, 1984
Assignee: E. I. Du Pont de Nemours & Co.

A method for the rapid determination of analyte in a sample is described. The sample is contacted with a solid phase having an analyte-analog immobilized, to which a labeled anti-analyte antibody is displaceably bound. Because the antibody has greater affinity for the analyte than the analyte–analog, the labeled antibody is displaced from the solid phase. The complex is separated from the solid phase, and the amount of complex is measured.

Gibbons, I., Rowley, G. L., and Ullman, E. F.
CHARGE EFFECTS IN ENZYME IMMUNOASSAYS
US 4,501,692, Feb. 26, 1985
Assignee: Syva Company

A method for determining a member of a specific binding pair ligand and receptor (antiligand). Reagents employed include a first modified member that provides an electrical field resulting from its plurality of ionic charges and a second labeled member. In the assay, the proximity of the first and second modified members is related to the amount of analyte; the observed label is related to the effect of the electrical field.

Gerber, B., Block, E., Bahar, I., Cantarow, W. D., Coseo, M., Eaton, C., Jones, W., Kovac, P., and Bruins, J.
ENZYME IMMUNOASSAY WITH TWO PART SOLUTION OF TETRAMETHYLBENZIDINE AS CHROMOGEN
US 4,503,143, Mar. 05, 1985
Assignee: BTC Diagnostics Limited Partnership

Colorimetric detection of antibodies and antigens, using chromogens of improved sensitivity and stability, is described. The chromogens take the form of activated solutions containing tetramethylbenzidine or its water soluble derivatives and are of particular use in home testing applications for detection of antigens.

Greenquist, A. C., and Walter, Bt.
HOMOGENEOUS SPECIFIC BINDING ASSAY DEVICE AND PREFORMED COMPLEX METHOD
US 4,442,204, Apr. 10, 1984
Assignee: Miles Laboratories, Inc.

A homogeneous specific binding assay device is described, determining a ligand, such as antigen or antibody, in a liquid sample. The test device consists of a solid carrier member and a fibrous web matrix incorporated with reagents for a homogeneous specific binding assay system, which produces a detectable response. The device consists of: (a) A reagent composition, including a complex of a labeled conjugate (ligand–label) and a specific binding partner for this ligand; and (b) a carrier incorporated with this complex.

Gordon, J., Staehelin, T., and Towbin, H.
ELECTROPHORETICALLY TRANSFERRING ELECTROPHEROGRAMS TO NITROCELLULOSE SHEETS FOR IMMUNOASSAYS
US 4,452,901, Jun. 05, 1984
Assignee: Ciba–Geigy Corporation

Solid supports are described, consisting of nitrocellulose sheets containing a replica of an electrophoretic separation of proteins in a gel. A faithful replica on the nitrocellulose support can be obtained by contacting the gel with a nitrocellulose sheet and applying an electric field perpendicular to the plane of the gel, causing an electrophoretic migration of the proteins toward the nitrocellulose sheet, where the proteins are adsorbed.

Harris, C. C.
CASCADE AMPLIFICATION ENZYME IMMUNOASSAY
US 4,463,090, Jul. 31, 1984

The invention describes enzyme immunoassays whose sensitivity is increased by cascade amplification. The coupled ligand (enzyme or an activator) catalytically activates a second enzyme, which acts on a substrate or can act on a third enzyme to produce a cascade. Alternatively, a proenzyme is coupled to the ligand and converted by an activator to an enzyme that is itself an activator of a second proenzyme in a cascade.

Hart, H.
TRANSPARENT OPTICAL CONTAINER FOR NONDESTRUCTIVE BIOLOGICAL FLUID ASSAY
US 4,451,434, May 29, 1984

Immunological assays are described in which two different classes of particles interact at short distances to produce characteristic detectable signals. An aqueous suspension of appropriately coated tritiated latex particles (LH) and polystyrene scintillant particles (L*) is employed. The amount of (LH) (L*) dimer formation and higher order aggregation represents the concentration of antibody (or antigen) present and can be determined by using standard liquid scintillation counting equipment and a specially designed optical container.

Henry, R. P.
NOVEL PHOTON ABSORBING OR EMITTING POLYMERS AND THEIR USE AS REAGENTS IN IMMUNOASSAY SYSTEMS
US 4,452,886, Jun. 05,1984

New polymers are described that absorb or emit photons in the visible or ultraviolet spectrum, which when bound to ligands, are useful as reagents for the detection, by immunoassay, of substances in physiological fluids.

Hirschfeld, S.
ASSAYING FOR A MULTIPLICITY OF ANTIGENS OR ANTIBODIES WITH A DETECTION COMPOUND
US 4,514,508, Apr. 30, 1985
Assignee: Biond Inc.

A universal method for the immunological determination of biological and pharmaceutical substances is described, which eliminates the need for multiple samples at multiple laboratories and minimizes the time required for such determinations. The assay is based on the recognition and binding of an antibody and an antigen or hapten to form a complex, and the changes that occur in the antibody conformation and chemical properties when such a complex is formed. A solid phase matrix, having qualitative and/or quantitative spectrum of antibodies, antigens, or haptens, is bound in each position, i.e., each sample well of a microtiter plate. The sample is then distributed to each of the positions forming an antigen–antibody complex. A detection compound is labeled or subsequently introduced and will recognize the formed complex and bind to the Fc portion of the antibody molecule. Following serial washings, routine detection methods are employed.

Jones, W., and Gerber, B.
ENZYME IMMUNOASSAY WITH STEP OF IMMERSING SAMPLE IN DEIONIZED WATER
US 4,525,452, Jun. 25, 1986
Assignee: BTC Diagnostics Limited Partnership

Immunoassay detection of bacterial diseases and microorganisms, using a deionized water collection medium. Enzyme immunoassay using colorimetric detection is described, which is useful in clinical or home testing applications for detection of bacteria, bacterial antigens, and antibodies against the bacteria.

Kamentsky, L. A.
IMMUNOASSAY METHODS EMPLOYING PATTERNS FOR THE DETECTION OF SOLUBLE AND CELL SURFACE ANTIGENS
US 4,487,839, Dec. 11, 1984
Assignee: Ortho Diagnostic Systems Inc.

Methods are described for determining the presence of antigens (or antibodies) in an aqueous sample or on the surface of cells, by using fluorescent antigens that compete with the sample antigens for antibody binding sites. The antibodies are deposited on a support surface means in alternating patterns and are detected using a translocated fluorescence detector. The signal, a repeating pattern of fluorescence, is analyzed by a gated integrator responsive to a gate track control means, also located on the surface means.

Kirkemo, C. L., and Shipchandler, M. T.
FLUORESCENT POLARIZATION ASSAY FOR LIGANDS USING AMINOMETHYLFLUORESCEIN DERIVATIVES AS TRACERS
US 4,510,251, Apr. 09, 1985
Assignee: Abbott Laboratories

A method is described for determining ligands in biological fluids by using a novel class of aminomethylfluorescein derivative, tracer compound reagents in fluorescence polarization immunoassays.

Langone, J. J.
IMMUNOASSAY UTILIZING $^{(125)}$I PROTEIN A
US 4,430,318, Feb. 07, 1984
Assignee: The United States of America, represented by the Department of Health and Human Services

An improved method is described for the preparation of $^{(125)}$I-labeled Protein A [$^{(125)}$I-PA] of high specific and functional activities. This $^{(125)}$I-PA has been used in combination with purified rabbit IgG bound to a solid support to develop a competitive binding assay capable of detecting Protein A or IgG at the nanogram level. An improved method of iodination of Protein A, using the Bolton–Hunter (1973) reagent, is described.

Lenhoff, H. M., and Ngo, T. T.
HETEROGENEOUS IMMUNOASSAY METHOD
US 4,506,009, Mar. 19, 1985
Assignee: University of California

A conjugate, having an immunoreactive component and an insolubilizing binding component that is also bound to a marker, is described, which is useful in determining the amount of antigen (or antibody) in a liquid sample. The insolubilizing binding component portion of the conjugate will react with an insolubilizing receptor to form a solid product of conjugate and receptor, unless the conjugate reacts with the corresponding antigen to be analyzed. The conjugate will be added to a liquid sample containing an unknown amount of, for example, an antibody. A known amount of the corresponding antigen is also added, which reacts with both the conjugate and antibody. After the reaction is

complete, the liquid sample is contacted with the insolubilizing receptor. Since only the free conjugate reacts with the insolubilizing receptor, the amount of antibody originally in the liquid sample can be determined by measuring the activity of the marker in the precipitate.

Litman, D. J., and Ullman, E. F.
SIMULTANEOUS CALIBRATION HETEROGENEOUS IMMUNOASSAY
US 4,533,629, Aug. 6, 1985
Assignee: Syva Company

An assay method is provided for determining the presence of an analyte that is a member of an immunological pair (mip), comprising ligand and receptor. By providing a first measurement surface capable of specifically binding a labeled reagent in an amount depending upon the presence of analyte in the sample and a second calibration surface capable of binding a second labeled reagent in a manner unaffected by the presence of analyte in the sample, calibration of individual tests can be accomplished simultaneously with the performance of the test itself. A signal producing system includes an enzyme bound to a mip that defines the first labeled reagent for binding to the measurement surface and the same enzyme conjugated to a ligand capable of binding to the calibration surface. Preferably, both labeled reagents have the same composition, and the calibration surface includes anti-(first enzyme).

Magnusson, C. G. M., Collet, C. D., and Masson, P. L.
IMMUNOASSAY OF PROTEINS
US 4,455,381, Jun. 19, 1984
Assignee: International Institute of Cellular and Molecular Pathology

In the immunoassay of a particular protein in a biological fluid, there is frequently interference in the assay by other proteins present in the fluid, e.g., by complement factors or antibodies in human serum. The interference so caused can be avoided by subjecting the fluid to protein-digestion, using, for example, an enzyme such as pepsin, a result of which, the particular protein of interest can be assayed without interference by the other proteins. Also, radioallergosorbent tests for particular IgE antibodies can be improved in sensitivity and accuracy by subjecting the absorbed IgE to enzymic digestion and then assaying a fragment thereof.

Marshall, D. L.
SOLUBLE INSOLUBLE POLYMERS IN ENZYMEIMMUNOASSAY
US 4,530,900, Jul. 23, 1985
Assignee: Seragen Diagnostics Inc.

An improved heterogeneous enzymeimmunoassay, involving the use of a reversibly soluble polymeric substance acting as the support for the antibody, is described. In the direct method, the antigen to be detected and

an enzyme labeled antigen are bound by antibody that is chemically linked to the soluble polymeric substance. The polymer is rendered insoluble and removed from the test solution. After resolubilization into a solution containing substrate for the enzyme label, the assay for antigen is completed by determination of enzymatic activity. In the indirect method, the antigen to be detected and an enzyme labeled antigen are incubated with a primary antibody unattached to the polymeric substance. After addition of a second antibody that is chemically linked to the polymeric substance, the polymer is rendered insoluble and the assay is performed, as in the direct method.

Masson, P. L., Collet, C. Dl., and Magnusson, C. G.
PARTICLE AGGLUTINATION IMMUNOASSAY WITH AGGLUTINATOR FOR DETERMINING HAPTENS; PACIA
US 4,427,781, Jan. 24, 1984
Assignee: International Institute of Cellular and Molecular Pathology

A particle counting assay for haptens is described, in which a liquid sample containing the hapten is mixed with finely divided inert particles bearing the same hapten, an agglutinator, such as RF or Clq, and a measured amount of antibody, the amount of which is insufficient to cause agglutination of all the particles. The amount of hapten is determinable by measuring the extent of the agglutination.

Murad, F., and Lewicki, J. A.
TWO-SITE IMMUNOASSAYS USING MONOCLONAL ANTIBODIES OF DIFFERENT CLASSES OR SUBCLASSES AND TEST KITS FOR PERFORMING SAME
US 4,474,892, Oct. 02, 1984
Assignee: Board of Trustees of The Leland Stanford Junior University

Two-site immunometric assays or multideterminant antigens are described, in which the antigen is reacted with an immobilized monoclonal antibody directed against one antigen determinant and a second monoclonal antibody that is directed against a distinct antigenic determinant and is of a different class or subclass than the immobilized monoclonal antibody. The second monoclonal antibody is either labeled (direct version) or is reacted with a labeled antibody against it (indirect version). The immobilizing medium and antibodies may be selected to reduce nonspecific binding and enhance sensitivity and/or permit signal amplification.

Neurath, A. R.
IMMUNOASSAYS USING SUPPORT-CONTAINING SEPARATE ANTIGENS AND ANTIBODIES DERIVED FROM AN IMMUNE COMPLEX
US 4,495,295, Jan. 22, 1985
Assignee: New York Blood Center, Inc.

A process is described for the detection of an antigen (or antibody) in a specimen by: (a) contacting the specimen with a support having bound to it a mixture of antigens and antibodies to antigen (or antibody) in the specimen (the antibodies and antigens bound to the support are separately bound to and not in the form of an immune complex) and incubating and washing the support; (b) contacting the washed support (a) with a radio- or enzyme-labeled antibody (or antigen), incubating, and washing; and (c) effecting radioimmunoassay or enzyme-labeled immunoassay.

Neurath, A. R., and Strick, N.
LABELED ANTIHAPTEN ANTIBODIES AND THEIR USE AS A UNIVERSAL REAGENT FOR SOLID PHASE RADIO- AND/OR ENZYMEIMMUNOASSAYS
US 4,495,296, Jan. 22, 1985
Assignee: New York Blood Center, Inc.

A process is described for detecting the presence of an antigen in a specimen by: (a) contacting the specimen with a support coated with antibodies to the antigen in the specimen, incubating, and washing the support; (b) contacting the support (a) with a hapten conjugated antibody against the antigen, incubating, and washing; (c) contacting the support (b) with a radio-labeled or enzyme containing antihapten antibody, incubating, and washing; and (d) effecting radioimmunoassay or enzyme-labeled immunoassay.

Neurath, A. R.
SENSITIVE IMMUNOASSAYS OF ANTIGENS OR ANTIBODIES SEQUESTERED WITHIN IMMUNE COMPLEXES
US 4,459,359, Jul. 10, 1984
Assignee: New York Blood Center, Inc.

The presence of an antigen (or antibody) in a sample, in the form of an immune complex, is determined by: (a) contacting the immune complex in the sample with a dissociating buffer to afford antigen and antibody; (b) contacting a solid support that binds proteins with the dissociated sample; (c) washing the solid support; (d) adding protein to fill unoccupied sites on the solid support; (e) adding radio- or enzyme-labeled antibody, incubating, and washing; and (f) measuring the radioactivity or enzymatic activity associated with the solid support.

Ozkan, A. N.
IMMUNOASSAY FOR DETERMINATION OF IMMUNE COMPLEXES WITH POLYMER-COATED PLASTIC BASE
US 4,450,231, May 22, 1984
Assignee: Biostar Medical Products, Inc.

An immunoassay of a serum is described to determine immune complexes. This assay is performed by producing a layer of a non-

proteinaceous, nonionic polymer that will adhere to the plastic base and has the capability of absorbing immune complexes of the specimen. A specimen is placed on the layer and treated to determine the amount of immune complexes. The polymer may be polyethylene (or an adduct) glycol, dextran, polyvinyl chloride, or a polymeric polyol. Washing is performed with conventional solutions, addition of an antihuman IgG coupled with an enzyme (or radiolabeled), and addition of a substrate (similar to ELISA) with color measurement by spectrophotometer.

Pauly, H. E., Kapmeyer, W., and Seitz, U.
LATEX, BIOLOGICALLY ACTIVE LATEX CONJUGATES, AND A PROCESS FOR THEIR PREPARATION
US 4,448,908, May 15, 1984
Assignee: Behringwerke Aktiengesellschaft

A latex, reactive with a biologically active substance to form a conjugate suitable for serological or immunological assay procedures, is described. The particles are composed of a latex polymer core and a shell with a water insoluble monomer of the formula: $[RCH_2COCHN_9(CH_2)_nCHOR'$, $R = CH_3$ or H, R' = alkyl or aryl, n = 1–6].

Reynolds, R. A.
AFFINITY IMMUNOASSAY SYSTEM
US 4,504,585, Mar. 12, 1985
Assignee: Aalto Scientific, Ltd.

An affinity immunoassay system, in which a solid phase non-immunological, group specific ligand is used to insolubilize the analyte of interest, either simultaneously, before, or after binding all of the analyte with a labeled monospecific antibody, and the concentration of the analyte is then determined by measuring the label activity present in the solid phase in relation to a single point calibrator solution of the analyte.

Schrenk, J.
HOMOGENEOUS ENZYME IMMUNOASSAY WITH HEATING STEP AFTER INCUBATION, THERESIA
US 4,510,240, Apr. 09, 1985
Assignee: AS Boehringer Mannheim GmbH.

A process is described for the determination of an antigen in homogeneous aqueous phase by incubation in the presence of antigen-specific antibodies and of a definite amount of enzyme-marked antigen and measurement of the activity of the marker enzyme. After incubation, the reaction solution is heated in the absence of the antigen-specific antibody under conditions that inactivate, by at least 50%, the marker enzyme and the enzyme activity measured.

Self, C. H.
IMMUNOASSAY USING AN AMPLIFIED CYCLIC DETECTION SYSTEM
US 4,446,231, May 01, 1984

An immunoassay is described, in which an enzyme label converts a precursor into a cycling factor, which in turn is interconverted in a cycling detection system. Detection is amplified by cycling NADP to NAD, with NAD cycling to NADH and back, preferred.

Smith, L. H., and Teplitz, R. L.
METHOD OF TESTING FOR PARTICULAR ANTIBODIES IN THE SERUM OF A PATIENT
US 4,493,899, Jan. 15, 1985
Assignee: City of Hope

A method is provided for testing for particular antibodies in serum. The antibodies (e.g., those of systemic lupus erythematosus) may constitute IgG and IgM immunoglobulins and may be individually radiolabeled. An antigen (e.g., DNA) may be attached to a support (e.g., by UV irradiation). The particular antibodies may be attached to the antigen and an assay performed to determine the attachment of the particular antibodies and attachment to the supported antigen.

Sun, M.
STABILIZED PEROXIDASE COMPOSITIONS
US 4,504,579, Mar. 12, 1985
Assignee: Abbott Laboratories

Novel stable peroxidase compositions containing a stabilizer, i.e., gentamicin, amikacin, and tobramycin, are disclosed, which are useful as reagents in enzyme immunoassay procedures.

Sokoloff, R., and Reno, J. M.
METHOD FOR REDUCING NONSPECIFIC INTERFERENCES IN AGGLUTINATION IMMUNOASSAYS
US 4,536,478, Aug. 20, 1985
Assignee: Seragan Diagnostics, Inc.

An agglutination immunoassay in which nonspecific interferences are reduced by adding a halogen substituted carboxylic acid to the reaction mixture.

Stryer, L., Glazer, A. N., and Oi, V. T.
FLUORESCENT IMMUNOASSAY EMPLOYING A PHYCOBILIPROTEIN-LABELED LIGAND OR RECEPTOR
US 4,520,110, May 28, 1985
Assignee: The Board of Trustees of the Leland Stanford Junior University

Sensitive detection techniques are described, employing fluorescent proteins having bilin prosthetic groups as labels. The bilin-containing proteins can be conjugated to ligands or receptors for use in systems involving ligand-receptor binding for the analysis, detection, or separation of ligands and receptors.

Szoka, F. C.
LIPOSOMES WITH GLYCOLIPID-LINKED ANTIBODIES
US 4,483,929, Nov. 20, 1984
Assignee: Liposome Technology Inc.

Lipid vesicles, labeled with encapsulated reporter compositions and bound to antibodies comprising a new class of immunoreagent, useful in immunoassays for ligands, are described.

Tom, H.
PERIODATE REMOVAL OF ASCORBATE INTERFERENCE IN DIPSTICKS FOR IMMUNOASSAYS
US 4,444,880, Apr. 24, 1984
Assignee: Syva Company

A solid support "dipstick" immunoassay impregnated with sodium metaperiodate eliminates ascorbate interference with an assay having a peroxidase signal producing system.

Tsuji, Y., and Wakabayashi, K.
LIGHT SCATTERING IMMUNOASSAY INVOLVING PARTICLES WITH SELECTIVE FREQUENCY BAND APPARATUS
US 4,446,239, May 01, 1984
Assignee: Chugai Seiyaku Kabushiki Kaisha

An immunoassay using a suspension of insoluble microscopic carrier particles, one type carrying an antigen (or antibody), forming an agglutination based on an antigen–antibody reaction. Irradiating the solution of the reaction system with laser light and detecting the light scattered from the reaction system at specific angles and at specific frequency bands can be used to calculate the quantity of antigen.

Unger, J. T.
IMMUNOASSAY FOR CLASS SPECIFIC IMMUNOGLOBULIN ANTIBODIES
US 4,434,227, Feb. 28, 1984
Assignee: Abbott Laboratories

A method is described for determining an immunoglobulin of the IgX class (X is M, A, D, or E). In a sample, Anti-IgG is added to IgG to prevent binding of rheumatoid factor before the sample containing IgX is added to insolubilized IgG.

Vander–Mallie, R.
USE OF ANTI-IDIOTYPE ANTIBODIES IN IMMUNOASSAYS
US 4,536,479, Aug. 20, 1985
Assignee: E. I. Du Pont de Nemours and Company

The use of anti-idiotype antibodies as functional substitutes for antigens or haptens in immunoassays, is described.

Wang, C. G.
METHOD OF TAGGED IMMUNOASSAY
US 4,454,233, Jun. 12, 1984
Assignee: Wang Associates

An immunoassay method is described for measurement of the content target antigen (or antibody) in a fluid or tissue specimen by reacting the target with reagent antibody to form a complex and is carried by small tagged mobile units having tagging elements. The tagged mobile units (latex particles smaller than 0.8 μm) bearing formed complexes are measured by spectroscopic detection. The tagged complexes that are formed may be measured by spectrophotometric detection or mass spectrometry. Different target antigens or antibodies can be assayed simultaneously by employing different tagged mobile units, and the mobile units with the tagging elements can be recovered for disposal or for reuse.

Wang, C. G.
TAGGED IMMUNOASSAY
US 4,436,826, Mar. 13, 1984
Assignee: Wang Associates

An immunoassay method is described for measurement of an antigen (or antibody) by reacting the target with reagent antibody, which forms a complex with the target and is tagged with tagging elements that are unassociated chemically with the reagent and are protected against reaction with the target. The reagent antibody is carried by small, tagged mobile units, such as latex particles (<0.8 μm). Different target antigens or antibodies can be assayed simultaneously by employing different tagged mobile units, and the mobile units with the tagging elements can be recovered for disposal or for reuse.

Wang, C. H. J., Stroupe, S. D., and Jolley, M. E.
FLUORESCENT POLARIZATION IMMUNOASSAYS
US 4,492,762, Jan. 08, 1985
Assignee: Abbott Laboratories

Fluorescent polarization immunoassays for substances in blood plasma or serum are conducted in dilute anionic surfactant solutions that disrupt the fluorescent bilirubin–serum albumin complex without disturbing the antibody reaction in the immunoassay reducing the background fluorescence.

Woods, J. W., St. Denis, J. N., and Chapman, D. A.
IMMUNOASSAY INVOLVING SOLUBLE COMPLEX OF SECOND ANTIBODY AND LABELED BINDING PROTEIN
US 4,469,787, Sep. 04, 1984
Assignee: Mallinckrodt Inc.

A method and kit is described for determining the presence of a polyvalent ligand by incubating with an immobilized antibody to form an immobilized ligand–antibody complex, incubating the complex with a solution of a soluble complex of a second antibody (to the ligand), and then specifically binding a labeled binding protein (such as protein A) to the Fc portion of the second antibody. The unbound second antibody and labeled binding protein are washed from the immobilized complex, and the presence of bound, labeled, binding protein is determined as a measure of the concentration of the ligand.

Zuk, R. F., and Litman, D. J.
IMMUNOCHROMATOGRAPHIC ASSAY WITH SUPPORT HAVING BOUND "MIP" AND SECOND ENZYME
US 4,435,504, Mar. 06, 1984
Assignee: Syva Company

Chromatographic immunoassay is described, which employs a specific binding pair member and a label conjugate that delineates a border whose distance from one end of the chromatograph relates to the amount of analyte present. An immunochromatograph is employed having both a specific binding pair member and an enzyme fixed to the support. A sample is chromatographed and the amount of analyte is determined by: (1) chromatographing a second enzyme conjugated with a specific binding pair member that binds to the chromatograph in proportion to the amount of analyte bound to the chromatograph; or (2) including the second enzyme conjugate with the sample itself, resulting in a defined border related to the amount of analyte in the sample. The two enzymes are related in that the substrate of one is the product of the other, so that upon contact of the chromatograph with appropriate reagents, a detectable signal develops that permits detection of the border to which the analyte traveled. This distance can be related to the amount of analyte present in the sample.

ENZYME ASSAYS AND ELECTRODES

Boguslaski, R. C., Carrico, R. J., and Christner, J. E.
HETEROGENOUS SPECIFIC BINDING ASSAY EMPLOYING AN ENZYME SUBSTRATE AS LABEL
US 4,492,751, Jan. 08, 1985
Assignee: Miles Laboratories, Inc.

A heterogeneous specific binding assay method is described that employs a reactant as a labeling substance in the detection of a ligand. The

method is carried out using a reagent containing, as its labeled constituent, a conjugate formed of a specific binding substance coupled to the reactant, such as an enzyme substrate or coenzyme, utilized as a means for monitoring the extent of binding. The presence of a ligand in a liquid medium may be determined by competitive binding. After the necessary separation of the bound and free phases, the extent of binding of the labeled constituent is determined by contacting either phase with the necessary materials to monitor the reaction system.

Carpenter, C. R., Dodge, R. H., and Rosanoff, K. A.
INACTIVATION OF ENZYMES
US 4,483,922, Nov. 20, 1984
Assignee: AMF Inc.

A method for inactivating enzymes, particularly enzymes found in human serum, by reacting the serum enzymes with an inactivating amount of peracetic acid. The treated serum is subsequently neutralized and dialyzed. This serum can be used as standards for calibration or as a control in assay kits.

Clark, L. C., Jr.
CUTANEOUS METHODS OF MEASURING BODY SUBSTANCES
US 4,458,686, Jul. 10, 1984
Assignee: Children's Hospital Medical Center

Cutaneous methods for measurement of substrates in mammalian subjects are described. A condition of the skin is used to measure a number of important substances that diffuse through the skin or are present underneath the skin in the blood or tissue. An enzyme whose activity is specific for a particular substance or substrate is placed on, in, or under the skin for reaction. The amount of the substrate is determined by measuring, for instance, the enzymatic reaction or by-product of the reaction detected directly through the skin. Polarographic electrodes or enzyme electrodes are employed as skin-contact analyzers in the transcutaneous measurement of oxygen or hydrogen peroxide to quantitatively determine blood substances, such as glucose and alcohol. In a preferred quantitative technique, the skin is arterialized when the measurements are made. Colorimetric detection methods are also employed.

Gorton, L. G., Jaegfeldt, H., Torstensson, A. B., and Johansson, G. R.
ELECTRODE FOR THE ELECTROCHEMICAL REGENERATION OF COENZYME, A METHOD OF MAKING SAID ELECTRODE, AND THE USE THEREOF
US 4,490,464, Dec. 25, 1984

Electrodes are described for the electrochemical regeneration of the coenzyme, NADH, (or analogs). The electrode surface has been modified by

adsorbing a condensed aromatic ring system with at least three condensed aromatic rings. Electrodes are prepared by adsorbing a condensed aromatic ring system to the surface of carbon or a graphitic material. Finally, the use of these electrodes for the regeneration of coenzyme is described.

Kaufman, R. A.
METHOD FOR INCREASING THE SENSITIVITY OF ASSAYS
US 4,451,563, May 29, 1984

Diagnostic reagents and a method for increasing the sensitivity of chemical and enzymatic analysis are described. An improved reagent containing a water-soluble inclusion compound increases the sensitivity of the analysis.

Nankai, S., Imai, A., and Iijima, T.
ENZYME ELECTRODE
US 4,431,507, Feb. 14, 1984
Assignee: Matsushita Electric Industrial Co., Ltd.

An improved enzyme electrode is described, which includes a first electrode having at least one kind of an enzyme immobilized on it for electrochemically detecting a substance to be produced in association with a reaction based on the enzyme and a second electrode for electrochemically removing materials that interfere with the detection by the first electrode.

Paulus, H. P.
BISPECIFIC ANTIBODY DETERMINANTS
US 4,444,878, Apr. 24, 1984
Assignee: Boston Biomedical Research Institute, Inc.

A homogeneous sample of identical bispecific antibody determinants is described in which each determinant is composed of two L–H half-molecules linked by disulfide bonds, each L–H half-molecule being specific for a different antigenic determinant, and including at least the F(ab') portion of a monoclonal IgG antibody. The bispecific antibody determinants are useful in the formation of multilamellar assemblies and enzyme electrodes.

Seago–Jame, S. L.
BIOLOGICAL DETECTION PROCESS USING POLYMER-COATED ELECTRODES
US 4,517,291, May 14, 1985
Assignee: E. I. Du Pont de Nemours and Company

A method is described for the amperometric determination of the concentration of a constituent of a biological sample that is a substrate for an oxidase enzyme. The enzyme catalyzes the consumption of oxygen and

the production of hydrogen peroxide, which can be measured by an inert metal indicator-electrode, coated with a thin film of a perfluorosulfonic acid polymer.

Tsuji, N., Nakamura, K., Endoh, K., Hamada, T., and Ishida, K.
SYSTEM FOR CONTROLLING A PRINTER IN A BLOOD SUGAR ANALYZER
US 4,483,924, Nov. 20, 1984
Assignee: Fuji Electric Company, Ltd.

A blood sugar analyzer is described, which has a reaction cell that houses a fixed enzyme membrane and measuring electrode. The analyzer receives a blood specimen, causing a chemical reaction between the specimen and membrane. The reaction current generated in the electrode by the reaction and temperature in the cell are monitored.

Literature

This section surveys the literature in the area of immunological and enzymatic assays published from January 1984 to September 1985. This section is not intended to be all encompassing and lists only some of the major articles and reviews that appeared during this time period.

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